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AN UNDESCRIBED SPECIES OF PAPULOSPORA PARASITIC ON RHIZOCTONIA SOLANI KUHN

JOHN R. WARREN

(WITH 3 FIGURES)

In an investigation to determine the cause of the black-root disease of sugar beets in Ohio, 690 isolations were made from diseased seedlings. Seventy or 7.6 per cent of the total number of isolations yielded *Rhizoctonia solani* Kuhn. As expected, the fragments of beet tissue from which *R. solani* was isolated frequently yielded cultures of other species of soil fungi. One of these, which appeared with greater frequency than any other species, was an organism subsequently recognized as an undescribed species of *Papulospora*. This fungus was isolated thirty times and it constituted 4.3 per cent of the total number of isolates. Although usually isolated from seedlings from which *R. solani* was also isolated, occasionally this *Papulospora* was the only fungus obtained from apparently diseased seedlings.

In the investigation of sugar beet black-root a series of tests were made to determine the pathogenicity of representatives of all species of fungi isolated. These tests included six isolates of *R. solani* and six isolates of the *Papulospora*. Although they were of varying degrees of virulence, all of the tested isolates of *R. solani* caused symptoms of the black-root disease. Two of the six isolates were extremely virulent and prevented the survival of any seedling beets growing in the infected soil. The number of seed-

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lings surviving ranged from twenty-five per cent to 100 per cent less than the number of seedlings in pots of soil free of the organism.

The pathogenicity tests made with the isolates of *Papulospora* were negative in that a stand of seedlings similar to that in the control pots was obtained and none of the symptoms characteristic of black-root were developed. There was, however, a marked stunting, or reduction in the rate of growth, of the seedlings growing in soil to which this organism had been added as compared with those growing in soil free of the fungus. When the stunted beets were removed from the soil and examined microscopically, there was no evidence that they had been invaded by the *Papulospora*.

Although the *R. solani* isolates were extremely virulent in pot tests, it is unusual for a field planting of sugar beets to be completely destroyed by black-root caused by this organism. This seemed to indicate either a limited occurrence of the fungus in the soil of the beet-producing area or an antagonistic condition between *R. solani* and other soil fungi. To determine if the latter possibility was involved, several species of fungi were tested to determine if a reaction occurred between them and *R. solani*. Among the fungi so tested was the species of *Papulospora* which had occurred so frequently in culture with *R. solani*.

METHODS

The pathogenicity tests of the two species of fungi were made by mixing ten day old cultures of the organisms with soil taken from sugar beet fields and steam sterilized. The cultures serving as inoculum were growing on a corn meal-sand medium. After the soil was mixed with the inoculum, six inch, unglazed pots were filled with the mixture and a moderate amount of water was added to the surface. Three days later the pots of soil were seeded with sheared seed of a type used in the commercial beet fields of Ohio. Twenty-five seed pieces were placed in each pot of soil and covered to a depth of one quarter inch. Control pots containing a mixture of sterilized soil and the corn meal-sand medium were prepared and maintained under the same conditions as the test pots.

In preliminary tests for antagonism between the fungi the organisms being tested were plated with *R. solani* on two per cent agar media in Petri dishes. The media used included potato-dextrose, prune, carrot, lima bean, and corn meal agars.

To determine the microscopic relationship between the two fungi when they grew together, slide cultures were made. For this purpose two large loopfuls of melted potato-dextrose agar or corn meal agar were placed in the center of flamed, vaseline-ringed cover slips. After the medium had cooled, hyphal transplants of the organisms were made from actively growing cultures to the opposite sides of the drop of medium and the cover slips were inverted over flamed, hollow ground slides.

To determine the behavior of the two fungi in the soil, microscope slides were buried in pots of previously sterilized soil which had been mixed with cultures of the two organisms growing separately on a corn meal-sand medium. Ten to fifteen days later the slides were carefully freed from the soil, air dried, and stained by immersion in carbol fuchsin maintained at 100 degrees centigrade in a water bath.

The effect of the presence of the *Papulospora* in reducing the amount of sugar beet root rot caused by *R. solani* was evaluated by a series of pot tests. In these tests ten day old cultures of each of the fungi growing separately on corn meal-sand medium were mixed with steam sterilized soil. Pots of soil containing *R. solani* alone were also established as were control pots. Twenty-five seed pieces were planted in each six inch pot of soil-culture mixture.

OBSERVATIONS AND EXPERIMENTAL RESULTS

When this species of *Papulospora* grew on the same plate of medium with *R. solani,* conspicuous results were obtained. Along the line of juncture of the colonies there developed, as shown (FIG. 1), a band of mycelial growth denser in character and whiter in color than that of other parts of the culture. With increasing age this band continued to widen until the area over which the *R. solani* had grown was covered with the dense, white growth.

A microscopic examination made in the region of the denser growth revealed that the mycelium of the *Papulospora* had over-

grown that of the *R. solani* and that the hyphae of the latter had become entwined by those of the *Papulospora* as shown (FIG. 2). Uniform coils were formed, the number on any one hypha increasing with the age of the culture. In cultures eight to ten days old complete ensheathment of the *R. solani* hyphae had usually resulted as shown (FIG. 3).

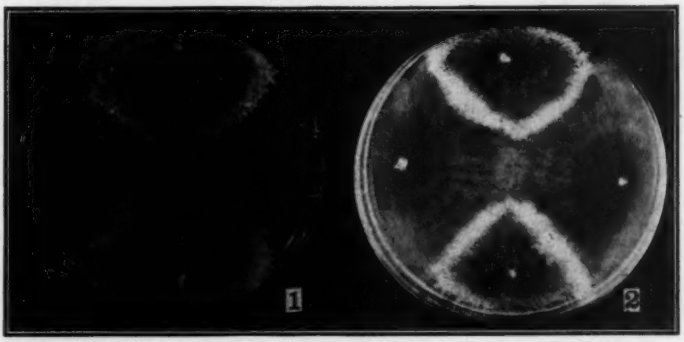

FIG. 1. Petri dish cultures of *Rhizoctonia solani* and *Papulospora stoveri*.

The cover slip-slide cultures showed that there was no apparent physiological attraction between the hyphae of the two fungi when they were separated from each other. After the hyphae, growing from opposite sides of the culture, had come into contact with each other the growth rate of the parasite was increased and the subsequent extension of the *Papulospora* paralleled the older hyphae of *R. solani*. Usually after the hyphae had been in contact for a distance of twenty to thirty microns lateral branches developed on the *Papulospora* hyphae. These grew rapidly, coiling around the hyphae of *R. solani*. In some instances less than ten minutes were required for the production of a single coil.

When a lateral branch of the *Papulospora* first began to form, the protoplasm of the *R. solani* hyphae separated into two parts each of which retracted from that part of the hypha nearest the developing branch. By the time several encircling branches had developed, the protoplasm of the host had disintegrated and only the walls of the hypha remained. Occasionally, instead of lateral

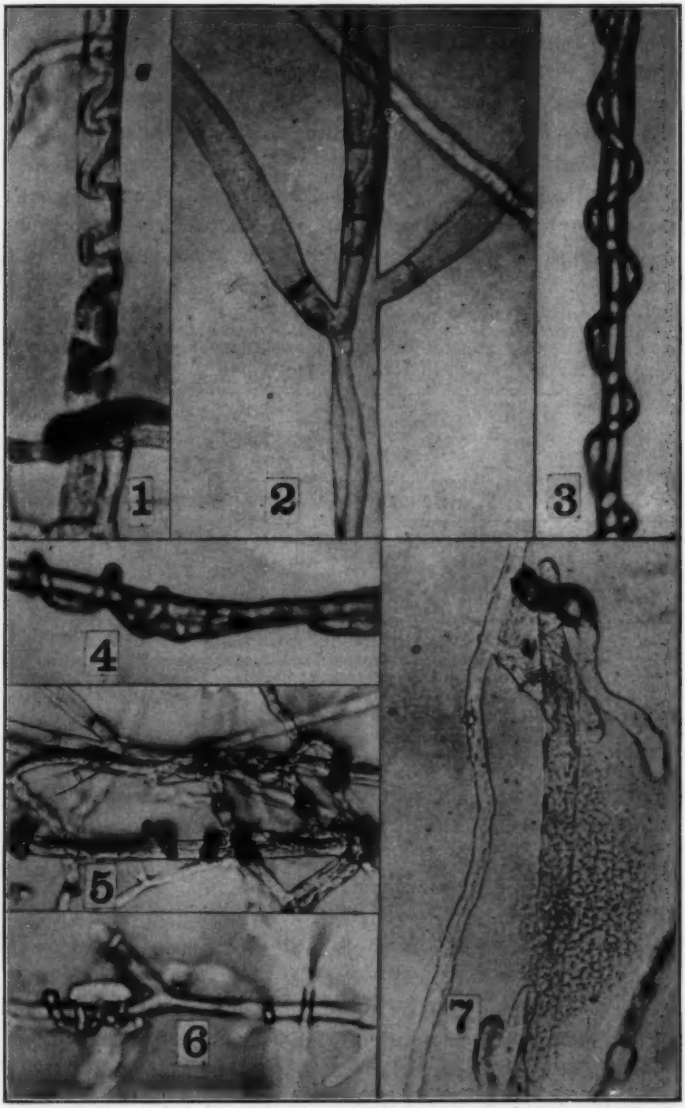

FIG. 2. Photomicrographs of *Rhizoctonia solani* parasitized by *Papulospora stoveri*.

branches developing, the *R. solani* hyphae were entwined by the *Papulospora* hyphae as the latter grew in length.

Although the interaction of the two fungi was predominantly as just described, it was not unusual to find numerous instances of the penetration of the *Papulospora* hyphae into those of *R. solani*. By removal of lengths of internally parasitized hyphae and examination with the aid of a micromanipulator it became apparent that the hyphae of the *Papulospora* had penetrated and were growing within those of *R. solani* as shown in figure 2.

TABLE I

THE EFFECTIVENESS OF A SPECIES OF *Papulospora* IN REDUCING THE AMOUNT OF SUGAR BEET BLACK ROOT CAUSED BY *Rhizoctonia solani* KUHN

Rhizoctonia solani Isolate Number	Number of Sugar Beet Seedlings Surviving in Soil Containing only *Rhizoctonia solani*			Number of Sugar Beet Seedlings Surviving in Soil Containing *Rhizoctonia solani* plus *Papulospora sloveri*		
	Series A[1]	Series B[1]	Series C[2]	Series A[1]	Series B[1]	Series C[2]
1–a	0	0	6.5	.5	0	11.0
A1–20	3.0	0	3.0	3.5	1.0	17.0
64–4	0	1	2.5	3.5	3.2	2.7
H–10	7.5	9.5	11.2	14.0	8.5	20.4

A. Seeds planted four days after cultures and soil were mixed.
B. Seeds planted twenty-four days after cultures and soil were mixed.
C. Seeds planted fifty-five days after cultures and soil were mixed.
[1] Average of seedlings in four pots.
[2] Average of seedlings in eight pots.

This species of *Papulospora* parasitized *R. solani* when cultivated on a wide variety of media; and by use of buried slides, as previously described, it was found that parasitism also occurred in the soil. The parasitism in the soil occurred in the same manner as in Petri dish or cover slip-slide cultures.

It remained to be determined by a series of pot tests whether *Papulospora* in the soil could effect a reduction in the amount of black-root of sugar beet seedlings caused by *R. solani*. Hence, ten day old cultures of *R. solani* were mixed with soil in the amount of one 400 ml. flask of culture to two six inch pots of soil. Eight pots of soil were prepared for each of the four isolates. One-half of these (four pots of soil-culture mixture for each isolate) were then mixed with ten day old cultures of the *Papulospora*. Four

days later, two pots from the four for each isolate of *R. solani* and two pots from the four for each isolate of *R. solani* plus the *Papulospora* were planted with twenty-five beet seed pieces to each pot. The results of this planting are shown under the heading, Series A of Table 1. The remaining pots of soil were watered at frequent intervals and planted with beet seed twenty-four days after the cultures and the soil were mixed. The results of this test are headed Series B in Table 1. Fifty-five days after cultures and soil were mixed, all of the pots were replanted. These results are shown in Series C of Table 1. In each of these tests the seedlings were examined for symptoms of the disease ten days after planting.

From the results of this test it appears that this species of *Papulospora* is effective in reducing the incidence of disease caused by some but not all strains of *R. solani*, and that the efficiency increases as the interaction time is increased.

DISCUSSION

In this study the observations made of Petri dish cultures, cover slip-slide cultures, and of slides buried in soil containing *R. solani* and the *Papulospora* indicate an actual parasitism of *R. solani* by the *Papulospora*. This type of parasitism is in many respects similar to that described by Weindling (**10**) of the parasitism of *Trichoderma lignorum* (Tode) Harz. on *R. solani*.

There are numerous publications dealing with antagonism among microorganisms but those concerned with the actual parasitism of one fungus by another one are comparatively meager. Types of parasitism have been reported by Zopf (**12**, 548–551) and by Reinhardt (**7**) and detailed studies have been made by Blochwitz (**2**) and by Ayers (**1**) on the parasitism of some of the Mucorales.

The results of the cultural studies indicate that although the *Papulospora* grows well alone in culture it does obtain some substances from *R. solani* when parasitism occurs. Indirect evidence of this is the plasmolysis and subsequent disintegration of the *R. solani* protoplasm. Somewhat more direct evidence is the close paralleling of *R. solani* hyphae by those of the parasite, the increased growth rate, and increased bulbil formation of the *Papulospora* after parasitism has occurred.

The evidence obtained from pot tests in this study with *R. solani* and *Papulospora* indicates that given a sufficient interaction time, the parasitism of *R. solani* on sugar beet seedlings is significantly reduced. These tests also indicate that there are some strains of *R. solani* which are little affected by the *Papulospora*.

This possibility of suppressing the growth of phytopathogenic fungi by microbiological activities has been suggested by several experimenters. Sanford and Broadfoot (**9**) have shown that root rot of cereals caused by *Ophiobolus graminis* Sacc. can be controlled by the activities of various soil inhabiting microorganisms. Sanford (**8**) and Millard and Taylor (**6**) explain the reduction of potato scab, when a green rye crop is plowed down, as being due to the increased activity of other soil microorganisms. Greaney and Machacek (**4**) found the pathogenicity of *Helminthosporium sativum* Pamm., King and Bakke, a root rotting agent of cereals, was determined in part by the activity of *Cephalothecium roseum* Cda. However, in each of these studies the effects observed are apparently due to the production of diffusible substances rather than to parasitism.

King and his associates (**5**) have experimented with the control of *Phymatotrichum omnivorum* (Shear) Duggar, the cause of a root rot of cotton, by altering the soil microflora with organic amendments. Brown (**3**) has indicated that root rot of watermelons caused by *P. omnivorum* may be reduced by the presence of *Trichoderma* spp. in the soil. Weindling and Fawcett (**11**) have suggested that the soil acidification which caused a reduction in the damping off of citrus seedlings might be due primarily to the increased parasitic, antagonistic, and competitive effects of other soil fungi.

The results obtained by these investigators indicate the possibility that future studies will demonstrate that the soil population of *Papulospora* may be increased and the interaction time required for the destruction of *R. solani* decreased by suitable soil amendments.

Since this species of *Papulospora* forms bulbils copiously in culture, it was sent to Dr. J. W. Hotson for examination because of his extensive knowledge of bulbiferous fungi. Dr. Hotson has informed the writer that this species has not previously been de-

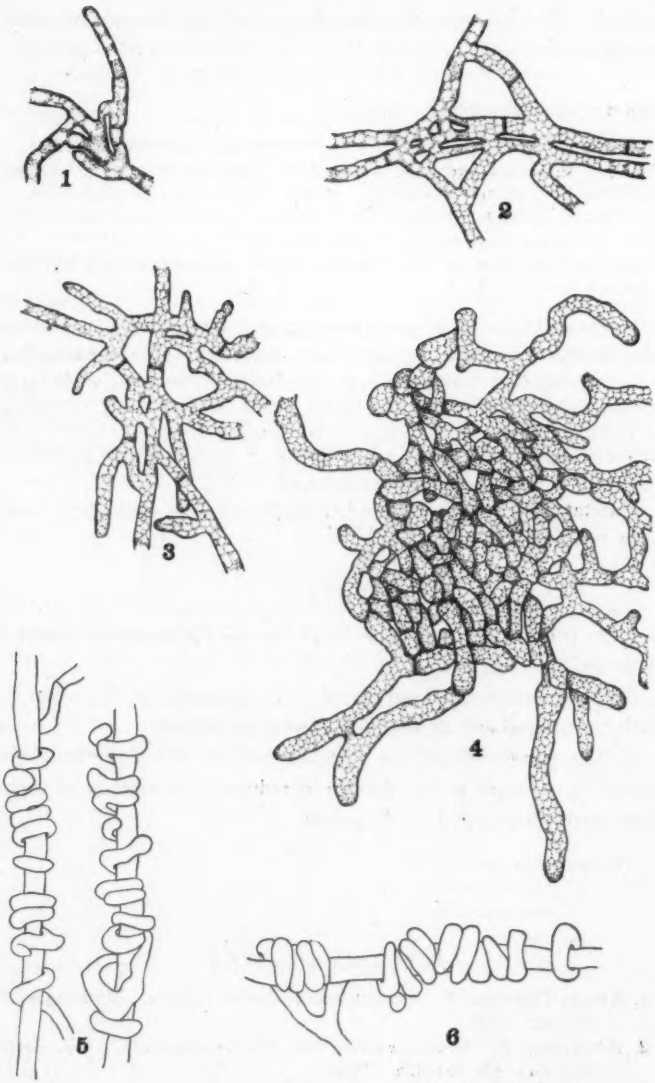

FIG. 3. Bulbil formation by *Papulospora stoveri*. 5 and 6, *Rhizoctonia solani* parasitized by *Papulospora stoveri*.

scribed. The description is as follows and the manner of bulbil formation is shown in figure 3.

Papulospora stoveri sp. nov.

Mycelium pallide luteum, procumbens, profusum; superficiei hyphis 5.7 μ diametro, submersis hyphis 6.7 μ diametro; inaequaliter septatis, ramosis, cum magnis globulis refractivis. Bulbilli colore maturi, rufo-brunnei; 630 × 488 μ, variabiles (480–640 × 384–488 μ); hyphae primordiales cellularum multarum intercalarum. Conidia absunt.

Hab. in argillosa terra et in radicibus *Betae vulgaris* novellis prope Fremont, Ohio. U. S. A.

Mycelium light buff, procumbent, profuse, growing in and on the medium. Surface hyphae 5.7 μ in diameter, submerged hyphae 6.7 μ in diameter, irregularly septate, much branched, with large refractive globules. Bulbils burnt sienna when young changing to reddish brown at maturity; size ranging from 480 × 384 μ to 640 × 544 μ. Average size 630 × 488 μ; primordium many intercalary cells. No conidia are produced.

Habitat. From clay soil and from the roots of sugar beet seedlings near Fremont, Ohio. U. S. A.

SUMMARY

1. A previously undescribed species of *Papulospora,* isolated from soil, is described.

2. This species has been found to be parasitic on *R. solani* by both an internal and an external type of parasitism.

3. The parasitism of the *Papulospora* on *R. solani* has been found, in pot tests, to be effective in reducing the amount of sugar beet black-root caused by *R. solani.*

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DESCRIPTION OF FIGURES

FIG. 1. Petri dish cultures of *Rhizoctonia solani* and *Papulospora stoveri*. *1. R. solani* alone, *2. R. solani* and *P. stoveri*.

FIG. 2. *Papulospora stoveri* parasitizing *Rhizoctonia solani*. *1–3*, × 600; *4–6*, × 300; *7*, × 450.

FIG. 3. *1–4*, Camera lucida drawings of bulbil formation by *Papulospora stoveri*. × 300. *5* and *6*, Camera lucida drawings of *Rhizoctonia solani* hyphae parasitized by *Papulospora stoveri*. × 300.

ENTOMOGENOUS FUNGI [1]

E. B. MAINS

(WITH 4 FIGURES)

CORDYCEPS PUIGGARII Speg.

In 1882, Spegazzini (**16**) under the name *"Cordyceps (Torrubia) sphaecophila* (Kl.) Berk. and Curt." [2] published a description of a fungus collected by J. Puiggari on *Polybia fasciata* in Brazil. Later, in 1889, he (**17**) decided that it was a new species for which he proposed the name *Cordyceps Puiggarii*. In 1919, Spegazzini (**18**) again employed the name *C. Puiggarii* for a fungus which he designated as a new species. It was based on two collections of J. Puiggari (*Nos. 141* and *154*) on a beetle, *Lystronchus* sp. It is evident from the descriptions that two different species are involved which therefore cannot bear the same name. Petch (**13**) has suggested that the collection on *Polybia* may be *C. sphecocephala* and those on *Lystronchus, C. curculionum*. The orientations of the perithecia and the sizes of the asci as given by Spegazzini raise questions concerning the identities of the collections. Through the kindness of Juan C. Lindquist, it has been possible to study the specimens in the Spegazzini Herbarium of the Museo de la Plata.

The collection on *Polybia fasciata* (*Herb. Speg. 1771*) is labeled *"Cordyceps Puiggarii* Speg. Typus." On an inner packet *"C. sphaecocephala* (Kl.) B. & Br." and *"C. Humberti* Rob." are written along with some notes and sketches. The collection consists of several broken clavae arising from the thorax of the insect

[1] Paper from the Department of Botany and the Herbarium of the University of Michigan. The cost of two extra plates has been paid by the Herbarium of the University of Michigan.

[2] The name *Cordyceps sphecophila* Berk. & Curt. evidently is an error. Berkeley and Curtis (**2**) give *Torrubia sphecophila* Tul. as a synonym. However, Tulasne (**20**) published it as *Torrubia sphecocephala* and referred to *Sphaeria sphecocephala* Klotzsch.

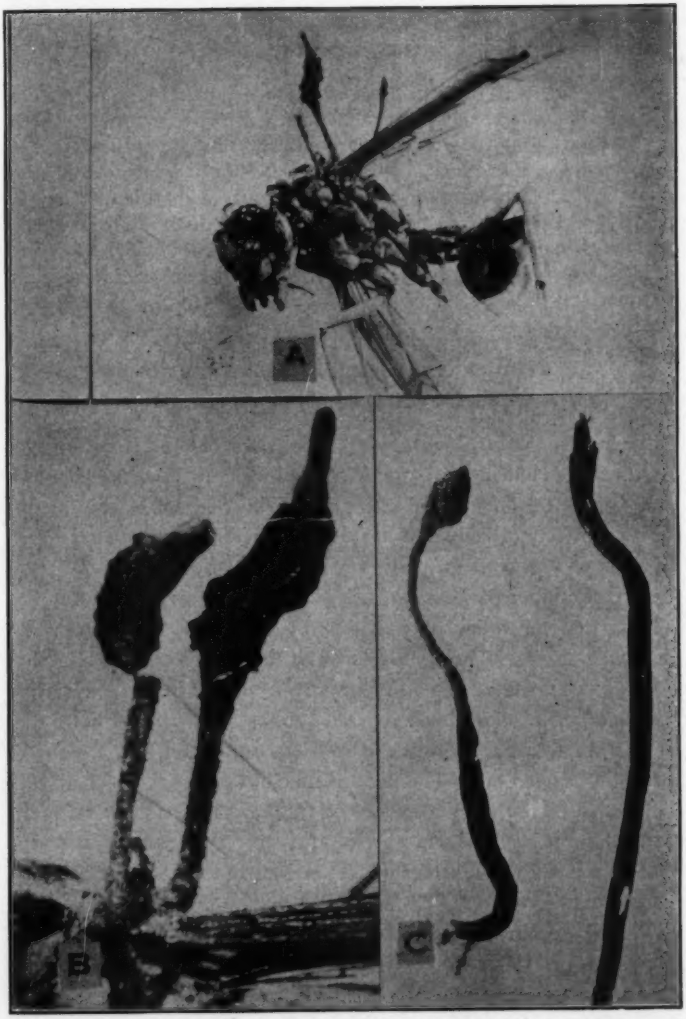

FIG. 1. *Cordyceps Puiggarii* Speg.

(FIG. 1, *A* & *B*). The fertile portions are broken off from all except two, and one is loose in the packet. The following data have been obtained from a study of the collection.

Clavae fusoid, 3–5 mm. long, the fertile portion 1–2 mm. long, 0.3–0.7 mm. thick, narrowed above into a sterile acuminate apex up to 1 mm. long, narrowed below into a stipe approximately 2–3 mm. long, 0.2 mm. thick, ochraceous-buff, the fertile portion olive due to the perithecia, the stipes noticeably puberulent specially below; perithecia embedded, prominent, producing an irregular surface, broadly ovoid to globoid, 320–420 × 300–320 μ, at right angles to the surface of the clava; asci fusoid-cylindric, narrowing to apex and base, 132–180 × 9–10 μ, the wall slightly thickened at the apex (2 μ); ascospores fusoid-cylindric, 90–110 × 2–2.5 μ overlapping in the ascus, multiseptate, not or tardily breaking into fragments. On *Polybia fasciata,* Apiahy, Brazil, V. 1881, Puiggari.

This is not *C. sphecocephala* which has oblique, much larger perithecia, cylindric asci up to 660 μ long and ascospores which early break into one-celled fragments. On account of similar differences it cannot be placed in synonymy with *C. oxycephala* Penz. & Sacc. as has been proposed by Kobayasi (**5**). From the notation on the inner packet, Spegazzini apparently considered the possibility that the collection might be *C. Humberti.* In general appearance it resembles *C. Humberti* as illustrated by Saussure (**15**). It also does not differ greatly in microscopic details from *C. Humberti* as described by Petch (**13**). Saussure did not figure sterile apices for the clavae. Petch has suggested that they were probably broken off. The puberulence of the stipes which is very noticeable in the type specimen of *C. Puiggarii* has not been described for *C. Humberti.* Apparently the two species are very closely related, but it seems best to recognize them as distinct from each other.

Of the specimens on the beetle, *Lystronchus* sp., *No. 141* of Puiggari (*Herb. Speg. 1773*) consists of fragments of a beetle and a short portion of a brown stipe. Specimen *No. 154* of Puiggari (*Herb. Speg. 1772*) consists of three fragments of stipes and two heads (FIG. 1, *C*). The clavae evidently were bicolored. The fragments of the stipes are brownish-black except for short portions of two which are a light yellowish-brown. The heads are

concolorous with the yellowish-brown portions of the stipes. The heads are fusoid-ovoid. The perithecia are narrowly ovoid, 780–960 × 216–300 μ, obliquely embedded and overlapping upward. The asci are narrowly cylindric, up to 480 μ long and 6–8 μ wide. Only immature ascospores were found. These collections are *C. curculionum*.

CORDYCEPS SUBSESSILIS Petch

Cordyceps subsessilis was described by Petch (**14**) from collections made by Roland Thaxter in North Carolina and Tennessee. The development of the fungus is somewhat unusual for a *Cordyceps* (FIG. 2 *A, Type*). Petch states "it would appear that the fungus produces perithecia as soon as its mycelium reaches the surface of the wood, the apparent stalk being merely the strand of mycelium in the wood, probably in the insect bore-hole."

This species appears to have an aberrant development of the stipe. Two collections made by A. H. Smith several years ago show a similar development. One of these (*14573*) arises from a white mycelial mass with fragments of an insect probably a beetle (FIG. 2, *C*). A stalk-like portion, which was 5 mm. long and 4 mm. wide when collected, is crowned by a poorly defined head consisting of vertical perithecia partly embedded in a white stroma. In the other collection (*7724*) the two heads terminate white stalk-like strands 5 mm. long and 0.5–1.2 mm. thick (FIG. 2, *B*). The heads form slight terminal enlargements 0.8–1.8 mm. wide. Most of the perithecia are embedded except for their apices. A few are covered only in the basal portion apparently due to the shrinkage of the stroma. In both of these collections only the groups of perithecia showed above the rotten wood. The heads are connected to the buried insects by stalk-like strands. The asci are very long and narrow, measuring 450–600 × 3.5–4 μ. The embedded perithecia and the long asci do not agree with the description given by Petch who describes the perithecia of *C. subsessilis* as free and glabrous and the asci as 240 μ long. Through the kindness of Dr. Rolf Singer it has been possible to study the specimens from the Farlow Herbarium cited by Petch. Although most of the perithecia of the type specimen appear to be free, in a

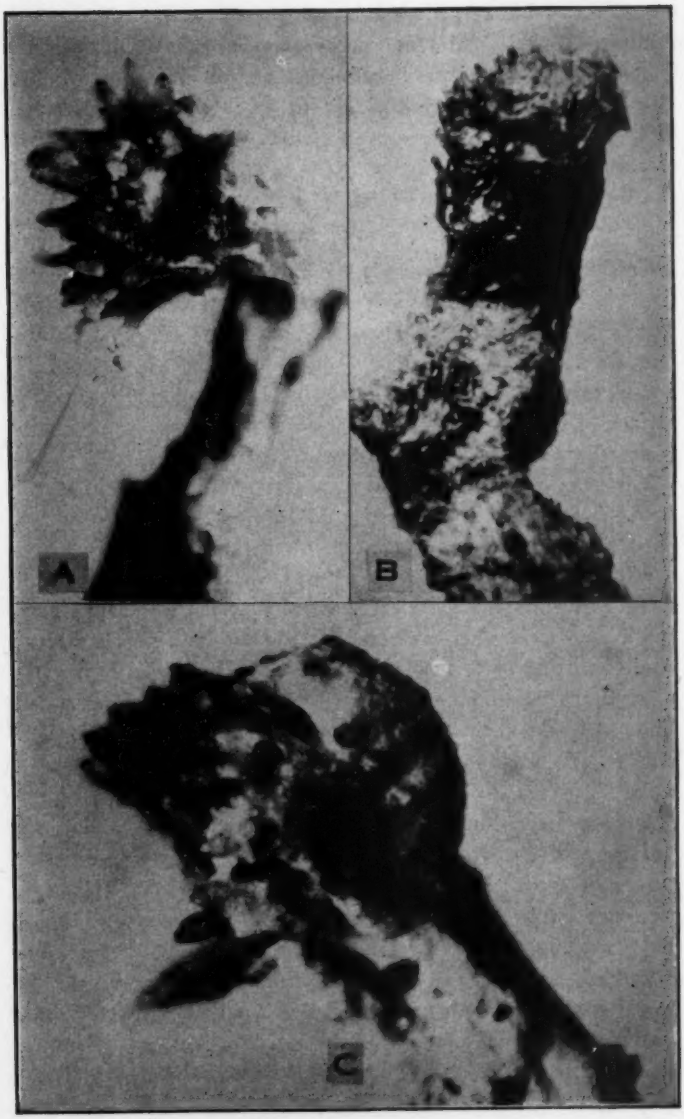

FIG. 2. *Cordyceps subsessilis* Petch.

small group they are covered except for their apices by a soft white stroma (FIG. 2, *A*). It would appear that typically they are covered at first and later become free by the shrinkage and disappearance of the stroma. The asci measure 430–540 × 4 μ. The following description is offered.

Clavae poorly developed, the stipes entirely included in the rotten wood of the substrata, developing irregularly, apparently reaching the surface through borer-holes from buried coleopterous larvae, brownish white with brown patches, 4.5–6 mm. long, 0.5–4 mm. thick, slightly enlarging on reaching the surface to form irregular heads 0.8–5 mm. wide, the heads at first white becoming light brown on account of the exposed perithecia; perithecia at first embedded in a soft stroma, later becoming mostly free and appearing superficial due to the shrinkage and disintegration of the stroma, light brown, conoid to narrowly ovoid, 900–1080 × 324–444 μ; asci narrowly cylindric, 430–600 × 3–4 μ, the ascospores filiform, breaking into part spores 3–6 × 0.5–1 μ.

On coleopterous larvae buried in rotten wood. Burbank, Tennessee. Aug. 1896, R. Thaxter (*Farlow Herb. No. 6145, type*); Cranberry, N. C., 1887, R. Thaxter (*Farlow Herb No. 6135*);[3] Drahner Road, Oakland County, Mich., Sept. 24, 1937, A. H. Smith (*7724*); Elwha River, Wash., June 22, 1939, A. H. Smith (*14573*).

In discussing the species Petch (**14**) refers to the stipes as a pseudostalk. He expresses some doubt whether the species should be referred to *Cordyceps* or to *Torrubiella*. In *Torrubiella* the perithecia develop in a stroma covering the host. It therefore would seem best to consider this a species of *Cordyceps* with an aberrant stipe.

CORDYCEPS PELTATA Wakefield

In 1916 Miss Wakefield (**21**) described a very interesting and unusual entomogenous fungus on *Cryptorhynchus* from the West Indies and named it *Cordyceps peltata*. Only the peltate heads were developed above the substratum. The stipes are described as short, entirely immersed in the substratum. The clavate asci contain fusiform multiseptate ascospores which, unlike ascospores

[3] Specimen consists of a few fragments and a number of loose perithecia.

of this type in other species of *Cordyceps,* separate at maturity into two halves. As far as I have been able to ascertain, this species has not been reported again. Several years ago it was noted that a specimen is deposited in the Farlow Herbarium from Barbados Island. Through the kindness of Dr. Rolf Singer it has been possible to study the specimen and prepare the following description.

Fertile portions of the fructifications peltate, closely appressed to the substratum, 2–3 mm. across, 0.7–1.0 mm. thick, coalescing to form an irregular series 10 mm. long, each apparently developing an umbo at the center, vinaceous-buff when immature becoming chestnut with vinaceous-buff margins at maturity, punctate on the upper surface from the slightly projecting ostioles of the perithecia; stipes short, up to 2 mm. long, completely embedded in the substratum, irregular, up to 1.5 mm. wide above, narrowing below, ochraceous-buff, arising from a brownish horizontal rhizomorph-like strand which develops from a dense white mycelial covering of the insect; perithecia vertical, opening on the upper surface of the head, ovoid, 430–480 × 168–204 μ; asci fusoid-clavate, attenuated below, narrowed above, 176–192 × 9–11 μ; the wall not noticeably thickened at the apex; the ascospores slightly overlapping in the upper portion of the ascus, fusiform, 72–84 × 3–4 μ, multiseptate, at maturity breaking into half-spores (FIG. 3, *A* & *B*).

On *Cryptorhynchus corticalis* Boh., Barbados, det. R. Thaxter (*Farlow Herb. 4046*).

The plant infested by the larva is not identified. Miss Wakefield states that the larva of her collection infested cultivated *Codiaeum.* The larva of the Barbados specimen occurs in a burrow and is surrounded by a dense white mycelial covering. From this a rhizomorph-like strand developed in the wood and produced progressively a number of fructifications at short intervals as indicated by variations in maturity (FIG. 3, *A,* from left to right). The crowding of the fructifications resulted in a coalescing of the peltate heads in an irregular line. Each head apparently produced a conical umbo in the center. All except the one on the youngest head to the extreme right are broken off, leaving only scars.

Lloyd (7) has commented on the unusual characters of this species and has questioned its inclusion in *Cordyceps.* He has suggested a close relationship to *Hypomyces* and *Hypocrea* and states

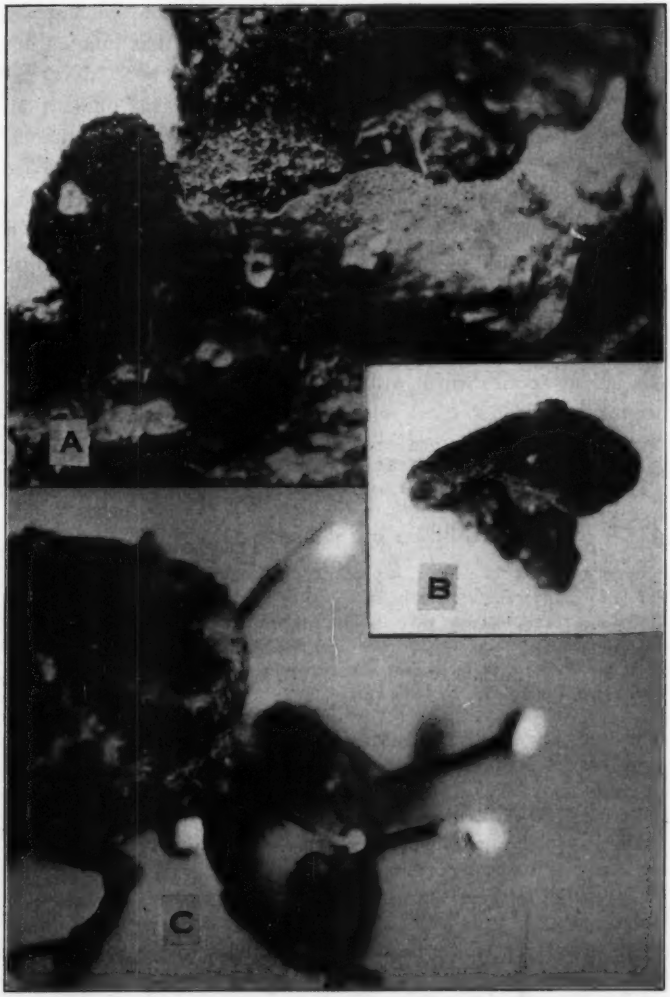

FIG. 3. *Cordyceps peltata* Wakefield.

that it "would be *Clintonella peltata* on spore character alone." Although the almost sessile heads might at first glance be mistaken for stromata of a *Hypocrea,* as Miss Wakefield has pointed out, this is only a superficial resemblance. Short, poorly developed stipes are formed. The long, narrowly fusiform, multiseptate ascospores do not resemble spore types of *Hypocrea.* A number of species of *Cordyceps* have similar spores and Petch (**10**) has separated these as a genus *Ophiocordyceps* including *C. peltata* as *Ophiocordyceps peltata* (Wakefield) Petch. The separation of the ascospores into half-spores is not found in other species of the group. The writer (**8**) has questioned the generic separation of the species of *Ophiocordyceps,* and Kobayasi (**5**) has recognized *Ophiocordyceps* as a subgenus of *Cordyceps. Cordyceps peltata* is a unique species which with our present knowledge of the genus cannot be placed in close relationship with other species. It appears to occupy an intermediate position between Ophiocordyceps and Eucordyceps types.

Stilbum burmense sp. nov.

Synnemata 1–3 mm. longa, capitata, capitulis globosis vel late obovoideis, 720–984 μ latis, pallide cremeis, stipitibus cylindraceis, 180–300 μ crassis, deorsum fuscoatris, sursum concoloribus capitulis; phialides densissime dispositae, anguste clavatae vel cylindraceae, 22–28 × 2–2.5 μ, apice unicum conidium gignentes; conidia hyalina, anguste obovoidea, 5–8 × 2 μ. Ex formica, Myitkyina, Burma, W. L. Jellison, 1945.

Synnemata arising from various parts of the body and appendages of the host, 1–3 mm. long, capitate, the heads globoid to broadly obovoid, 720–984 μ broad, light cream color, the stipes cylindric, 180–300 μ thick, brownish black below, concolorous with the heads in the upper portions, consisting of parallel, longitudinal hyphae, the inner colorless, 2.5 μ thick, thin-walled and sparsely septate, the outer in the lower portion of the stipe brownish, 4 μ thick and with septa 8–30 μ apart; phialides forming a very dense peripheral layer of the head, at right angles to the surface, narrowly clavate to cylindric, 22–28 × 2–2.5 μ; conidia hyaline, covered with a mucus, adhering to form a covering over the head upon drying, narrowly obovoid, 5–8 × 2 μ, smooth, produced singly at the apices of the phialides.

On a flying ant, Myitkyina, Burma, W. L. Jellison, 1945.

This interesting specimen was received from Edward A. Steinhaus.

The cream colored portions of the bicolored synnemata were probably some shade of red when fresh. *Stilbum formicarum* Cooke and Massee which also has bicolored synnemata was described (**3**) on an ant from Australia. It was described as having black stipes and obovate, roseous heads. Petch (**12**) has studied the specimen in the Kew Herbarium and states that the synnemata are linear with the apices acute, obtuse or slightly inflated into a head. He describes the conidia as broadly clavate or obovate, 6–9 × 2.5–4 μ, and the phialides as cylindric or clavate, 15–18 × 3 μ, apparently forming a palisade layer over the synnemata. Petch reaches the conclusion that *S. formicarum* is a *Hymenostilbe* and is a synonym of *H. melanopoda* Petch. The smaller spores, the definitely capitate synnemata and the limitation of the phialides to the head distinguish *Stilbum burmense*.

The bicolored synnemata of *S. burmense* strongly suggest that it may be the conidial stage of one of the species of *Cordyceps* of ants having bicolored clavae. *Cordyceps australis* (Speg.) Sacc., *C. bicephala* Berk., *C. necator* Pat. and Har., *C. proliferans* P. Henn. and *C. Huberiana* P. Henn. have been described as producing bicolored clavae on ants. Petch (**11**) states that *Hymenostilbe melanopoda*[4] is the conidial stage of *Cordyceps bicephala*. This conclusion is apparently based on the following statement by Spegazzini (**17**) concerning *Isaria melanopus*, "Species statum conidicum Cordicipitis australis Speg. facillime sistens." Spegazzini gives the hosts of *I. melanopus* as Coleoptera and it would seem very doubtful that it would be the conidial stage of a parasite of ants. A connection with *Cordyceps curculionum*, a bicolored species infecting beetles, would appear to be a more likely possibility. For a similar reason it is doubtful that *Stilbum formicarum* is synonymous with *Hymenostilbe melanopus* (*H. melanopoda*). Since the combination *Hymenostilbe formicarum* is preempted (Petch, **12**) the name **Hymenostilbe australiensis** nom.

[4] Petch (**11**) in transferring *Isaria melanopus* Speg. to *Hymenostilbe* changed the specific name, proposing the combination *Hymenostilbe melanopoda*. This is not justified and the combination should be *Hymenostilbe melanopus*.

nov. (*Stilbum formicarum* Cooke and Massee) is proposed. Both *Hymenostilbe australiensis* and *Stilbum burmense* are probably conidial stages of bicolored species of *Cordyceps* infecting ants. Until they are found associated with their ascigerous stages their specific connection will remain uncertain.

Mycologists are not in agreement concerning the application of the generic name *Stilbum*. Tode (**19**) proposed the name in 1790 for a genus, describing six species, the first being *Stilbum vulgare*. Fries (**4**) in 1832 in his treatment of *Stilbum* in his Systema Mycologicum included twenty-two species. The first species described is *Stilbum hirsutum*. *S. vulgare* is the nineteenth. Fries placed the genus in his third class, Hyphomycetes. For many years *Stilbum* has been used for a genus of the Hyphomycetes and more than 100 species have been described (140 included by Saccardo according to Ainsworth and Bisby, **1**). In 1900, Lindau (**16**) placed *Stilbum* in the Basidiomycetes near *Pilacre* including only one species *S. vulgare*. For the remaining species in the Hyphomycetes he proposed the name *Stilbella*. *Stilbum* as published by Fries (**4**) was unquestionably a genus of the Hyphomycetes, apparently with *S. vulgare* as the only exception among the species. It continued to be treated as such without question until 1900 with the addition of many species. Lindau's assumption that *S. vulgare* should be the type of *Stilbum*, resulting in the application of the name to a monotypic genus of the Basidiomycetes, does not appear to be justified if recommendation VI of the International Rules of Botanical Nomenclature (Cambridge revision) is followed.

STILBUM RAMOSUM Peck

In 1937, A. H. Smith collected a fungus on a lepidopterous larva near Ann Arbor. This has many of the characters of a *Stilbum*, differing principally in the branched polycephalous synnemata. In 1874, Peck (**9**) described a branched entomogenous fungus as *Stilbum ramosum*. His description is not sufficient to determine accurately the identity of the fungus. Through the kindness of H. D. House, the type collection in the Herbarium of the New York State Museum was loaned for study. It apparently is only

a portion of the original collection. It consists of two fragments of synnemata glued to paper (FIG. 4, *A*). They are 6 and 15 mm. long and 0.2–0.5 mm. thick. The synnemata have branches 6–7 mm. long with short secondary branchlets terminated by globoid to subgloboid heads, 0.3–0.8 mm. in diameter. From the fragments it is not possible to determine the arrangements of the

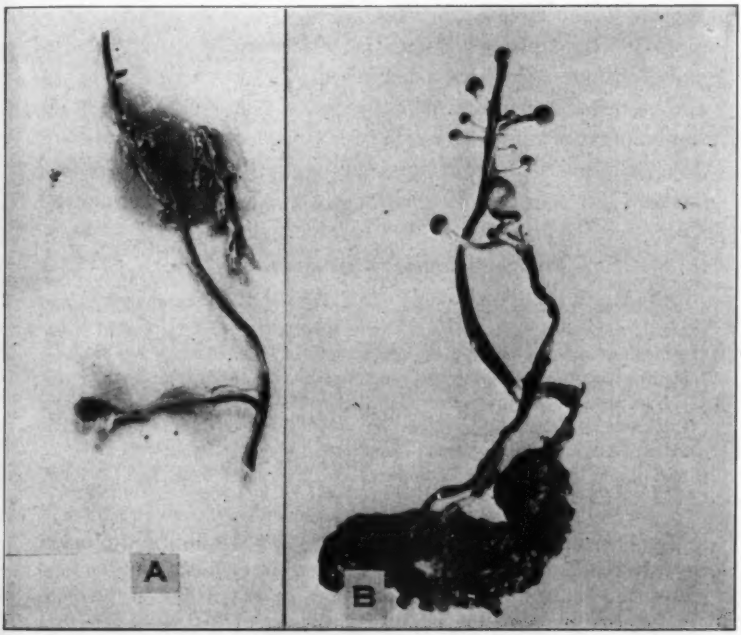

FIG. 4. *Stilbum ramosum* and *Polycephalomyces paludosus*.

branches. Peck states "sometimes creeping and sending up branches at intervals." The synnemata are now cinnamon-brown to light cream. Peck describes them as white above, pallid or brownish below. The heads are light brown and are covered by an agglutinated layer of spores. The conidia are hyaline, ellipsoid to obovoid, 2.2–3.3 × 1.1–1.5 μ and are covered by a mucus. They are produced singly on subulate phialides which are 15–30 μ long and up to 1.5 μ wide at the base. The phialides are closely com-

pacted forming a dense peripheral layer of the head. No part of the host was found with the type. Peck states that the fungus occurred on dead larvae of insects buried in rotten wood. This suggests that the host may be coleopterous.

Kobayasi (**5**) has described a somewhat similar species, *Polycephalomyces formosus,* on coleopterous larvae in Japan. His illustration shows stouter synnemata with shorter, more numerous, crowded to cespitose branches. The genus *Polycephalomyces* proposed by Kobayasi differs from *Stilbum* principally in the polycephalous synnemata, a distinction which has been used in separating other genera of the Stilbaceae. The combination **Polycephalomyces ramosus** (Peck) comb. nov. is proposed for *Stilbum ramosum.* The Michigan collections of Dr. Smith differ from both *P. formosus* and *P. ramosus* and a new species is proposed.

Polycephalomyces paludosus sp. nov.

Synnemata capitata, 10–20 mm. longa, 0.5–0.8 mm. crassa, cinnamomea, ramosis recte dispositis, 1–4 mm. longis, 0.1–0.2 mm. crassis; partes fertiles terminales, globosae, capitulis globosis, 0.2–0.4 mm. diam., flavo-brunneis; phialides capitulorum dense dispositae, subulatae, 12–20 μ longae, deorsum 1–1.5 μ crassae, apice unicum conidium gignentes; phialides ramorum sparsae, ventricosae, sursum raro stellatae, 10.5–14.7 × 1.5–2 μ; conidia hyalina, obovoidea, 1.8–2.5 × 1.1–1.3 μ, muco obducentia.

Ex larva lepidopteri, Kent Lake, New Hudson, Mich., Sept. 13, 1937, A. H. Smith (*7560*).

Synnemata capitate, 10–20 mm. long, 0.5–0.8 mm. thick, cinnamon-brown, branched, the branches at right angles, 1–4 mm. long, 0.1–0.2 mm. thick, the branches and the upper portions of the stems slightly pulverulent, the hyphae of the stipes 2–3.4 μ wide, thin-walled, hyaline and parallel in the interior, brownish and more or less interwoven in the outer layer; fertile parts terminating the main stem and branches, globoid, 0.2–0.4 mm. dia. including the dried layer of spores, yellowish brown, composed of hyaline, thin-walled hyphae radiating outward from the apex of the branch, repeatedly branching to form the dense peripheral layer of the conidiophores, the terminal phialides subulate, 12–20 μ long, 1–1.5 μ wide at the base, phialides occurring scattered on the branches below the heads, ventricose, occasionally stellate above, 10.5–14.7 × 1.5–2 μ; conidia produced singly, hyaline, obovoid, 1.8–2.5 × 1.1–1.3 μ covered by a mucus, agglutinating (FIG. 4, *B*).

On a lepidopterous larva, Kent Lake, New Hudson, Mich., Sept. 13, 1937, A. H. Smith (*7560*–**type**).

This collection was found closely associated with specimens of *Cordyceps paludosa* Mains (**8**) indicating that this may be the conidial stage of that species.

In addition to the difference in its host, *Polycephalomyces paludosus* has smaller conidia and more regular development of its synnemata than *P. formosus* and *P. ramosus*.

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EXPLANATION OF FIGURES

FIG. 1. *A, Cordyceps Puiggarii* Speg., type (*Herb. Speg. 1771*) on *Polybia fasciata* described in 1889. × 4. *B, Cordyceps Puiggarii,* type, showing puberulent stipes and sterile apex of clava. × 16. *C,* Two fragments of specimen 154 of Puiggari (*Herb. Speg. 1772*) described as *C. Puiggarii* by Spegazzini in 1919 (= *C. curculionum*). × 5.

FIG. 2. *Cordyceps subsessilis* Petch, *A.* One clava of the type (*Farlow Herb. 6145*); the perithecia mostly free with a few still embedded in a soft stroma. × 16. *B.* One clava of collection *Smith 7724,* the perithecia almost completely embedded in the stroma. × 16. *C.* Collection *Smith 14573,* part of the perithecia embedded and part free. × 16.

FIG. 3. *A. Cordyceps peltata* Wakefield (*Farlow Herb. 4046*) showing coalesced heads viewed from above. × 10. *B. C. peltata,* side view of one peltate head showing short stipe. × 12. *C. Stilbum burmense* showing bicolored synnemata arising from the head and thorax of an ant. × 6.

FIG. 4. *A.* One portion of the type of *Stilbum ramosum* Peck. × 5. *B. Polycephalomyces paludosus* Mains, type, showing polycephalous branched synnemata. × 3.5.

STATUS OF THE RUST GENERA ALLOPUCCINIA, LEUCOTELIUM, EDYTHEA, AND YPSILOSPORA [1]

M. J. THIRUMALACHAR AND GEORGE B. CUMMINS

(WITH 5 FIGURES)

The following notes have resulted from a careful study of specimens of the genera *Allopuccinia, Leucotelium, Edythea,* and *Ypsilospora.* For reasons stated below three of these genera are here reduced to synonymy.

ALLOPUCCINIA Jackson and LEUCOTELIUM Tranzschel

In 1931, Jackson (**5**) described the genus *Allopuccinia* to accommodate a rust on *Amicia lobbiana* from San Felipe and Sorata, Bolivia (Holway *611;* type). The genus was characterized as having subcuticular pycnia, subepidermal, uredinoid aecia (primary uredia) with peripheral paraphyses, and subepidermal telia with hyaline, stipitate, two-celled teliospores (FIG. 2) germinating without a rest period with the production of four-celled external basidia. The presence of subcuticular pycnia, a type not found in *Puccinia,* provided the principal character used in distinguishing *Allopuccinia* from *Puccinia.*

Sydow (**10**), in 1930, described the genus *Sorataea,* also on *Amicia lobbiana* collected by Rusby at Sorata, Bolivia in 1886. Uredia and telia were described. A comparison of the description of *Sorataea amiciae* Syd. with that given by Jackson for *Allopuccinia diluta* Jacks. & Holw. clearly indicates that both authors were describing the same fungus. Since *Sorataea* was published before *Allopuccinia* the latter genus becomes a synonym of *Sorataea* and *A. diluta* a synonym of *S. amiciae.*

[1] Journal Paper Number 323, of the Purdue University Agricultural Experiment Station. Contribution from the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin, and the Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

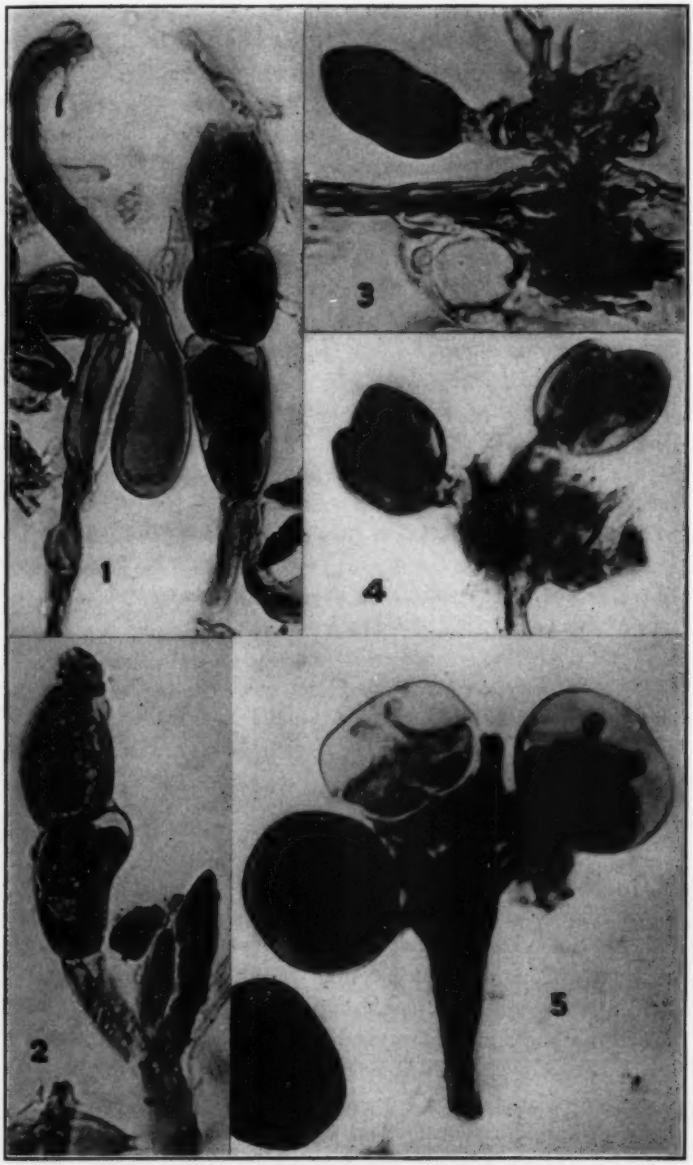
In our study of the sori we have found that, in the uredia and telia, the spores are borne in groups on basal sporogenous cells (FIG. 2), a feature not described by Sydow or Jackson but pointed out by Mains (8) in his study of the genus *Maravalia*. The presence of such basal cells has been used increasingly as a differential character in the separation of genera in the Uredinales although Kuhnholtz-Lordat (6) considers, in the genus *Puccinia*, that basal cells merely represent one method of teliospore development.

The importance accorded to the formation of basal cells will largely determine the validity of the genus *Leucotelium* Tranz. (13). In *Leucotelium* the teliospores are stipitate, two-celled, colorless, and develop singly from a compact hymenial layer. The pycnia in *Leucotelium* are also subcuticular but the aecia are aecidioid and the species [*L. cerasi* (Bereng.) Tranz.] heteroecious. Since the type of life cycle and the morphology of the aecia are not considered of significance in the delimitation of genera, the absence of basal cells remains as the only character separating *Leucotelium* from *Sorataea*. Until additional species are described in and more information is available concerning these two genera, it appears inadvisable to reduce *Leucotelium* to synonymy.

Three-celled teliospores (FIG. 1) have been found to occur in *Sorataea amiciae* which may indicate, as Jackson suggested (5), that *Sorataea* and *Mimema* are closely related.

EDYTHEA Jackson

Jackson (4), in 1931, described the genus *Edythea* to accommodate three South American rusts of *Berberis*, *E. quitensis* (Lagerh.) Jacks. & Holw., *E. berberidis* (Lagerh.) Jacks. & Holw., and *E. tenella* Jacks. & Holw. The principal characteristic of the genus is the presence of superficial uredia and telia, and the individual spores pedicellate from the apex of sporogenous stalks (FIG. 4) which emerge from the stomata prior to sporulation. The teliospores are nearly colorless, two-celled, mostly diorchidioid, and germinate without a rest period by the production of four-celled, external basidia. There is only a relatively small mass of hyphae beneath the stomata (FIG. 3). To quote Jackson: "There is no sorus in the usual sense of the term."



FIGS. 1–5. Spores of plant rusts.

Only a few sporogenous stalks emerge from the stomata and the stomata are not ruptured (FIG. 3). Sori of this type have been called extrastomatal by Cummins (1) and superstomatal by Mains (7). Pycnia and aecia are not known for the genus.

In 1918, the Sydows (11) described the genus *Desmella* and later Cummins (2) published an account of the morphology of the sori of *D. aneimiae* (P. Henn.) Syd. and *D. superficialis* (Speg.) Syd. The sorus in *Desmella* is superstomatal with a few sporogenous stalks which emerge from the stomata and bear, at their apices, several pedicellate spores (FIG. 5). The teliospores are diorchidioid, two-celled, and pale yellowish or hyaline. Pycnia and aecia are not known for any of the species.

It is obvious that the characters of *Edythea* duplicate those of *Desmella*. In the presence of such morphological similarity there is no justification for segregating genera on the basis of host groups, even if, as in the present case, the hosts are not closely related. Consequently we reduce *Edythea* to synonymy and propose the following transfer of species: **Desmella quitensis** (Lagerh.) n. comb. (*Uropyxis quitensis* Lagerh.; Arthur, Bot. Gaz. **65**: 464. 1918; *Edythea quitensis* Jacks. & Holw.; Jackson, Mycologia **23**: 99. 1931); **Desmella berberidis** (Lagerh.) n. comb. (*Sphenospora berberidis* Lagerh.; Arthur, Bot. Gaz. **65**: 464. 1918; *Edythea berberidis* Jacks. & Holw.; Jackson, Mycologia **23**: 99. 1931); **Desmella tenella** (Jacks. & Holw.) n. comb. (*Edythea tenella* Jacks. & Holw.; Jackson, Mycologia **23**: 100. 1931).

YPSILOSPORA Cummins

The genus *Ypsilospora* was described by Cummins (3) in 1941 for a leguminous rust (*Y. baphiae* Cumm.) collected on *Baphia nitida* in Sierra Leone (*Deighton 2138;* type). The rust has subcuticular pycnia and subepidermal telia. The teliospores, described as one-celled and borne in pairs at the apex of a common pedicel, are uniformly thin-walled, hyaline, and germinate without a rest period by the production of four-celled, external basidia. Some resemblance to the genus *Sphenospora* was noted.

After a careful re-examination of the rust we question the valid-

ity of the genus. The teliospores are developed in subepidermal sori and appear to be binate. The spores are of approximately the same size and, while often closely appressed to one another, there is no common wall. In younger pairs one of the spores is obviously smaller than the other, as indicated in the original illustrations (**3**). Further study has also revealed that the two spores are of unlike age; one of each pair germinates before the other.

These observations indicate that the structures originally described as pedicels should be interpreted as elongated basal sporogenous cells, each bearing two sessile teliospores that are produced successively and not simultaneously. This has also been suggested as a possible interpretation by Olive (**9**), in connection with his studies of *Sphenospora kevorkianii* Linder. Similar elongation of a basal cell with consequent similarity of appearance to a pedicel has been pointed out by Thirumalachar (**12**) in *Chrysocelis ascotela* (Syd.) Thirum. This interpretation of the morphology of the telia and teliospores together with the presence of subcuticular pycnia leads us to conclude that *Ypsilospora* is synonymous with *Chaconia* Juel. Consequently, we make the following generic transfer: **Chaconia baphiae** (Cumm.) n. comb. (*Ypsilospora baphiae* Cumm. Bull. Torrey Club **68**: 47. 1941) and consider the rust to be a microcyclic species of *Chaconia* in which only two teliospores, rather than several, develop on each basal cell.

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EXPLANATION OF FIGURES

FIG. 1. *Sorataea amiciae;* one paraphysis and one of the three-celled teliospores which occasionally occur in this species. FIG. 2. *Sorataea amiciae;* a typical two-celled teliospore attached to the basal cell. (Both from type of *Allopuccinia diluta* Jacks. & Holw.) × 800. FIG. 3. *Desmella tenella;* free-hand section through a telium. Note the small amount of substomatal mycelium, the basal cell passing outward through the stoma without rupturing the epidermis. FIG. 4. *Desmella tenella;* a single detached basal cell with two teliospores attached. (Both from type of *Edythea tenella* Jacks. & Holw.) × 800. FIG. 5. *Desmella superficialis;* a single detached basal cell bearing three teliospores. (From Thaxter *No. 46*) × 975. All preparations stained.

BASIC FUCHSIN AS A NUCLEAR STAIN FOR FUNGI [1]

EDWARD D. DELAMATER, M.D., Ph.D.[2]

(WITH 1 FIGURE)

Negative or weak Feulgen reactions have been obtained in many plant organisms. The three procedures for staining with basic fuchsin presented in this paper were developed in consequence of failure to obtain adequate Feulgen reactions in cytologic studies on certain fungi pathogenic for man, and because of the difficulty encountered in obtaining iron alum hematoxylin preparations which do not have a murky aspect. Clear and beautiful preparations can be obtained by the following technics, which appear to hold tremendous promise for studies of nuclear phenomena in the fungi.

Ohlmacher (**1895**) was the first and, we believe, the only author to use formaldehyde as a mordant for basic fuchsin staining. He applied his methods to bacteria and tissues. The studies of DeLamater and Ulrich extend, but only partially confirm, the initial observations of Ohlmacher.

A full account of the studies performed in the delineation of these procedures is presented elsewhere. These studies demonstrate that preliminary acid hydrolysis of the cells to be stained, comparable to that required in the Feulgen reaction, is necessary to increase the specificity of the stain for the nucleus. These studies also indicate that an aqueous solution of basic fuchsin produces an impermanent stain which, although extremely useful in wet mounts of tissue, fades in a short time. The stain can be made permanent, however, by (1) the mordanting of hydrolyzed cells in 1 to 4 per cent solution of formalin prior to staining or (2) by

[1] Read at the meeting of the American Association for the Advancement of Science, Chicago, Illinois, December 26 to 31, 1947.

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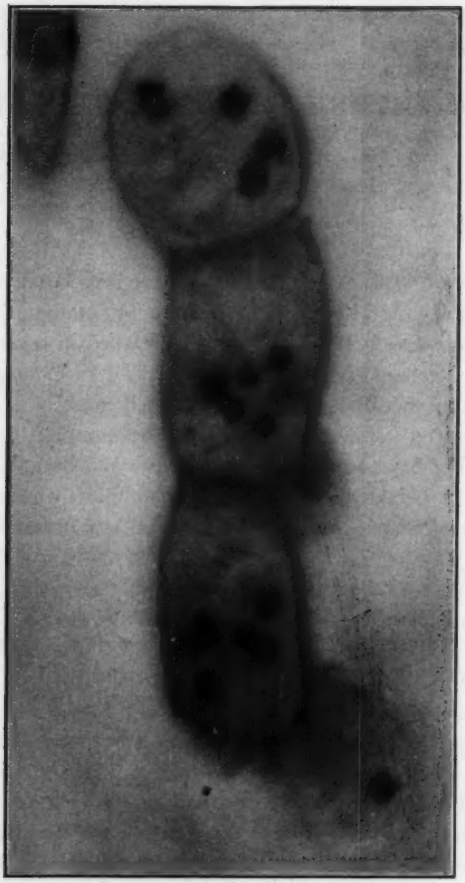

FIG. 1. *Blastomyces dermatitidis.* Cell grown at 37° C. on dextrose nutrient agar, showing early resting nuclei (formaldehyde-basic fuchsin stain, × 3,400).

the combining of 2 per cent formalin with the stain. It was shown further that the acid hydrolysis is as important to the mordanting effect of the aldehyde as it is to the specificity of the stain for the nucleus. Other dyes of the phenyl methane group, such as crystal violet, in which the para-amino radicals are blocked out with methyl groups, stain, but are not mordanted with aldehyde.

Materials and Methods

Preparation of solutions.—Basic fuchsin supplied by the National Aniline Division of the Allied Chemical and Dye Corporation with the certification number NF54 has been used. The dye was prepared as a 0.5 per cent solution in distilled water; to each 5 cc. of this solution 0.2 cc. of normal hydrochloric acid sometimes was added. The resultant solution has an approximate normality of twenty-fifth normal solution of hydrochloric acid. The dye also was prepared as a 0.5 per cent solution in twenty-fifth normal hydrochloric acid. Other solutions of basic fuchsin used will be described when the procedures are outlined. The normal hydrochloric acid for hydrolysis was prepared by dilution of concentrated (36 normal) hydrochloric acid with distilled water to the proper dilution. Alcohols of the proper dilution were prepared from 95 per cent and absolute alcohol by the addition of the proper amount of distilled water. Alcohols were used in 10 per cent stepwise dilutions. Only distilled water was used in the washing of cells after hydrolysis and after aldehyde mordanting.

Organisms tested.—Fourteen strains of *Blastomyces dermatitidis* from various sources, as well as *Candida albicans, C. albicans* variety *tropicalis, C. albicans* variety *stellatoidea, C. Krusei, C. parakrusei, C. guilliermondi, Histoplasma capsulatum* and *Saccharomyces* sp., were used as test organisms. Tissue sections from spontaneous *Blastomyces dermatitidis* infection in the dog also were used as test objects. Smears of material from mice infected with *Sporotrichum schenckii* were studied also. The most extensive studies to date have been done with *Blastomyces.*

Cultural methods.—The yeastlike phase of *Blastomyces* was induced by growing the organisms at 37° C. on a highly nutrient medium (dextrose nutrient agar). Cultures were fixed in situ with Schaudinn's fixative (Feulgen's and Zenker's fixatives were found to produce more shrinkage), suspended with a platinum loop and poured into 15 cc. centrifuge tubes. All manipulations of fungi in this phase were done in these tubes. Solutions were changed by centrifuging the cells, pouring off the old and adding the new solution. The filamentous phase of these fungi was induced at a temperature of 30° C. on a nutrient-poor medium, such

as cornmeal-extract agar or Czapek's agar in Petri dishes. Over the surface of the agar sterilized cellophane membranes cut to the size of a Petri dish were spread. The inoculation was made on top of the membranes. By regulating the amount of nutrient in the agar the thickness of the mycelial mat can be controlled. This can then be fixed in situ and manipulated through the various procedures to be described by simply transferring the cellophane membrane containing the fungus from one Petri dish to another containing the desired solution. At the time of mounting the mycelium is peeled from the membrane and mounted in pieces in clarite or balsam.

As stated all the three procedures for the use of basic fuchsin as a nuclear stain require carefully controlled acid hydrolysis comparable to that used in the Feulgen technic. A description of the three procedures follows.

Procedure 1: use of aqueous basic fuchsin.—Aqueous basic fuchsin without mordanting produces a precise, delicate and intense, red stain of the nuclei. This stain fades rapidly in water mounts, but is extremely useful for immediate direct observation. It may be made more lasting by mounting the cells in a sugar-acacia-glycerine mixture. Mounts made in this medium will last several weeks and are likewise extremely useful.

Fix cells in Schaudinn's solution ..... 1 hour
Wash: In 30 per cent alcohol .....15 minutes
In 20 per cent alcohol .....15 minutes
In 10 per cent alcohol .....15 minutes
In distilled water .....Wash
Hydrolyze in normal hydrochloric acid:
60° C. .....10 minutes
(Optimal hydrolysis periods should be determined for each organism studied. Temperature must be accurately controlled.)
Wash in distilled water ..... 5 minutes

Place in a 0.25 per cent aqueous solution of basic fuchsin (not acidified) for five to fifteen minutes to stain. (If samples of cells are observed during this period, the avidity of the nuclei for the dye can be observed and the staining stopped at any desired level.)

Wash in distilled water. (Destaining occurs in the wash water. The cells can be studied immediately, on wet mounts sealed with

vaselin. If cells are too heavily stained, continued washing will eliminate the excess.)

Water menstruum for mounts.—Lee has described the following aqueous mounting medium which has been found to give excellent preparations.

Solution A

This solution contains:

50 per cent solution of glycerin	2 parts
Cold saturated solution of sugar	1 part
Cold saturated solution of gum arabic	1 part

Four dilutions of this solution of varying strengths are prepared with distilled water and the cells are run through them before being placed in the solution of full strength. Too rapid exposure to the full strength solution tends to cause shrinkage or collapse of the cells. Should too great destaining occur during processing, more of the stain can be added at any point and the cell nuclei again will take up the dye.

Solution B

This solution contains:

Picked gum arabic (or acacia)	50 gm.
Cane sugar (not candied)	50 gm.
Distilled water	50 cc.

Dissolve over a water bath and add 0.05 gm. of thymol.

This solution may be diluted with solution A, and cells may be transferred through graded mixtures of the two solutions. Such a procedure helps to prevent collapse of the cells. The walls of *Blastomyces* and other fungi appear to act as selective semipermeable membranes. This action on the part of the cell walls can be extremely bothersome in cytologic work.

Procedure 2: use of aqueous basic fuchsin with aldehyde mordanting.—The aqueous basic fuchsin stain just described can be made permanent by mordanting the hydrolyzed cells in 2 per cent solution of formalin for two to four minutes before exposing them to the stain. The outline of the procedure follows:

Fix cells in Schaudinn's solution	1 hour
Wash: In 30 per cent alcohol	15 minutes
In 20 per cent alcohol	15 minutes
In 10 per cent alcohol	15 minutes
In distilled water	15 minutes

Hydrolyze in normal hydrochloric acid:
60° C. ..... 10 minutes
(Optimal hydrolysis periods should be determined for each organism studied. Temperature must be accurately controlled.)
Wash in distilled water ..... 5 minutes

Mordant in 2 per cent formalin (to make up: dilute formalin, 10 per cent solution of formaldehyde, to a 2 per cent solution with distilled water) for two to four minutes. Wash in distilled water. Stain in 0.5 per cent aqueous basic fuchsin in twenty-fifth normal hydrochloric acid for fifteen minutes. (As indicated previously this can be approximated sufficiently closely and without causing too great dilution of the stain, by adding 0.2 cc. of normal hydrochloric acid per 5 cc. of 0.5 per cent aqueous solution of basic fuchsin.)

Wash in distilled water.

Run through graded alcohols. Destaining occurs gradually in alcohols and can be observed directly. Rapidity of passage through the alcohols determines the degree of destaining. Acid alcohol can be used also for destaining, but it has proved difficult to stop the effect at the desired point with material being handled in test tubes. Once the proper degree of destaining is achieved the cells can be passed more rapidly through the remaining alcohols. Destaining is stopped entirely in xylene, in which basic fuchsin is insoluble.

Clear in xylene.

Mount in balsam or clarite.

Procedure 3: use of aqueous basic fuchsin with aldehyde mordant incorporated in the staining solution.—This method differs from the preceding only in the manner in which the stain is made up and in that no individual mordanting process is required. We have found the previous method easier to control, as a precipitation may occur in the stain when this method is used (FIG. 1).

The stain is made up as follows, so that it is 0.5 per cent basic fuchsin, 2 per cent formalin in a solution of a twenty-fifth normal hydrochloric acid.

1 per cent solution of basic fuchsin	50 cc.
Formalin (10 per cent solution)	20 cc.
Normal hydrochloric acid	4 cc.
Distilled water	26 cc.

Except for the fact that the mordanting process as outlined in procedure 2 is left out because the mordant is incorporated in the staining solution, the procedure differs in no way from procedure 2. The outline of the previous procedure will suffice for both.

Counterstaining.—Fast green can be used for counterstaining if desired. It does, however, tend to cover and obscure somewhat the delicate nuclear detail observable without its use. Fast green stains cell walls intensely and aids by defining the cells.

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THE NUCLEAR CYTOLOGY OF BLASTOMYCES DERMATITIDIS [1]

EDWARD D. DELAMATER, M.D., Ph.D.[2]

(WITH 5 FIGURES)

In the extensive literature pertaining to the fungi pathogenic for man and animals no reference has been found to the nuclear structure or mechanism of *Blastomyces dermatitidis,* Gilchrist and Stokes, 1898. The various articles dealing with this organism emphasize, for the most part, its morphologic characteristics (**2**, **3**, **15**).

Emmons (**10**) in a personal communication has admitted having observed the multinucleate status of this fungus, but has not, or had not at that time, followed up his observation. We (**6**, **7**) recently have evaluated cytologic technics and methods usable on this and other medically important fungous pathogens, and in addition have developed new technics, at least part of the chemical mechanism of which is understood. In these extensive studies *Blastomyces dermatitidis* (fourteen strains), *Histoplasma capsulatum,* the various species of *Candida* and *Saccharomyces* sp. have been used as test organisms. Comparable staining reactions have been obtained for all of these fungi with only slight modifications of technic for each. *Coccidioides immitis* presents certain difficulties and studies of this fungus will be reported separately elsewhere.

These studies of methods confirm Emmons' observation that *Blastomyces dermatitidis* is multinucleate.

Blastomyces dermatitidis gives a weak, but definitely positive, Feulgen reaction. This reaction, other than to give a positive, specific test for the presence of desoxyribose-nucleic acid in minute amounts in the several nuclei within the cell, is not usable as a cytologic technic for the study of nuclear phenomena. The stain-

[1] Read at the meeting of the American Association for the Advancement of Science, Chicago, Illinois, December 26 to 31, 1947.

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ing effect is much too weak and delicate to permit clear-cut observations. It is sufficiently strong, however, to permit chemical studies. Demonstration of the presence of desoxyribose-nucleic acid, even in minute amounts, is, however, of importance as additional evidence that thymonucleic acid is widely distributed among the fungi. This reaction also aided in the demonstration of the multinucleate condition of the cells of *Blastomyces*.

The multinucleate condition obtaining in *Blastomyces* deserves especial emphasis. In the presence of the demonstration by Hansen (**12**) of the so-called dual phenomenon and the additional work by Pontecorvo (**16**) and others (**17**) on heterokaryosis, the multinucleate condition assumes double importance as a basis for further experimental study of this fungus.

The size of the nuclei in many fungi has proved a barrier to elucidation not only of their structure (**1, 9**) but also of the sequence of events during their division. As yet unresolved discrepancies, for example, those between the recorded observations of Guilliermond (**11**) and of the Lindegrens (**14**) on the structure and division of yeast nuclei, emphasize the difficulties due to size of the nuclei, even though new methods, such as phase microscopy, have become available.

It seems important that the mechanism of division of chromosomal elements be established in organisms in which it is intended to study variation or genetics. It also seems important to know whether the particular mechanism involved follows the general pattern which has been established for other and larger forms, or whether new mechanisms are to be found.

It is the purpose of this paper to record observations made on what appear to be the structure and sequence of events during nuclear division and growth in *Blastomyces dermatitidis*. There are many gaps in the records due primarily to the minuteness of the objects being studied. In this study both old and new technics have been used and the results obtained by each have been remarkably consistent.

ORGANISMS STUDIED

Fourteen strains of *Blastomyces dermatitidis* from various sources have been studied. These fourteen strains showed mor-

phologic differences, one from another, which will be considered at length elsewhere. The nuclear phenomena observed in each strain were, however, comparable; no differences were noted either grossly or in detail. The presentation in this article, because similar observations were made on all fourteen strains, consists of a composite; the drawings (FIGS. 1–2) and photographs (FIGS. 3, 4 and 5) have been made from several of the strains.

METHODS

The organisms were grown in the yeastlike phase at 37° C. on dextrose nutrient agar. Cultures were fixed in situ, stirred into suspension with a platinum loop and poured into 15 cc. test tubes. All procedures on this phase of growth were carried out in these tubes. At the time of mounting, cells were mixed in the mounting medium, a drop of which was placed on a slide and a cover-slip was added.

Growth in the filamentous phase was handled in two ways. In the first, cultures were grown on Czapek's or cornmeal extract agar at 30° C. Growth on these media is thin but ordinarily an abundance of conidia and other structures are produced.

Such cultures were fixed in situ and blocks of agar were cut out and carried to water. From these the surface growth was carefully sliced with a sharp scalpel. These thin slices of agar containing the growth on their upper surfaces were stained with iron alum hematoxylin stain and mounted upright in balsam on slides. Such procedures were carried out in Petri dishes.

The second procedure consisted of growing the organisms on cellophane sheets cut to the size of Petri dishes, sterilized and placed on the surface of dextrose nutrient agar, cornmeal extract agar or Czapek's agar. These cultures were incubated at 30° C., fixed in situ and the colonies transferred to fresh Petri dishes. All staining procedures were carried out on these colonies in Petri dishes. At the time of mounting the mycelium was removed from the sheet and mounted upright on a slide in balsam or clarite.

By both these procedures the normal relationships of fungous structures could be maintained in preparations in which the cytologic picture could be easily studied.

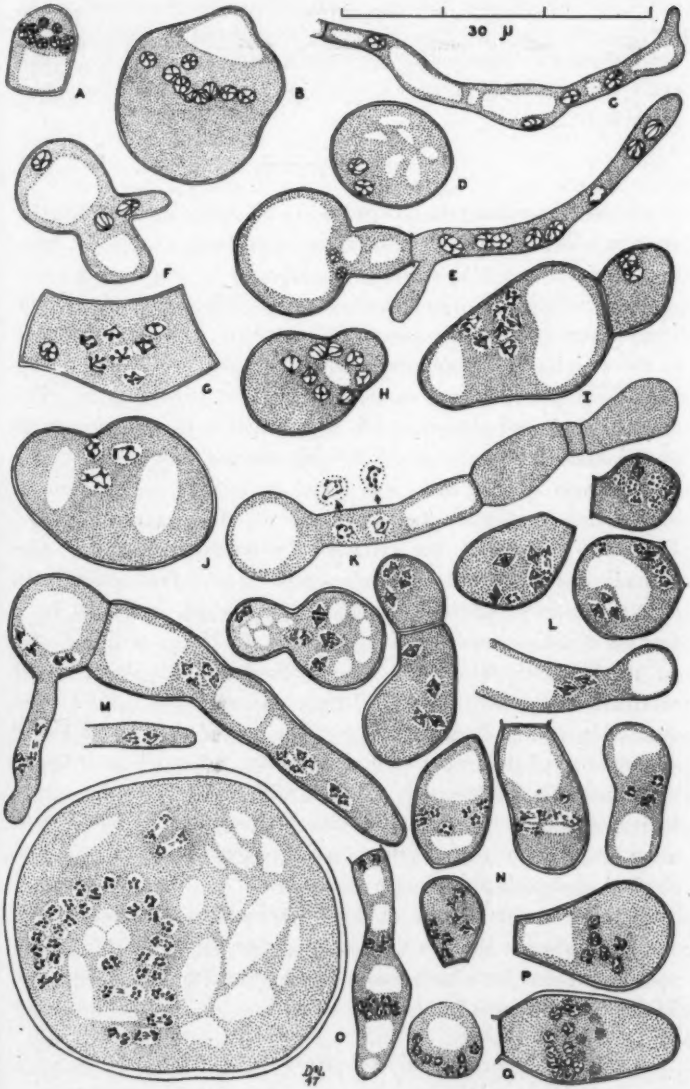

Fig. 1. *Blastomyces dermatitidis.*

FIXATION

Fixation was done with Schaudinn's solution at 60° C. Feulgen's and Zenker's fixatives were found to produce too great shrinkage.

STAINING PROCEDURES

The first procedure used consisted of an iron alum hematoxylin stain in which destaining was accomplished with a saturated solution of picric acid. Destaining was stopped by washing in water to which a drop or two of ammonium hydroxide were added. Iron alum hematoxylin preparations, however, have a cloudy aspect due to the staining of cytoplasmic elements which obscure the nuclei.

The Feulgen stain was carried out in the classic manner. The reader is referred elsewhere (**6**, **7**) for details of the procedures used both with this and the following staining procedures.

The basic fuchsin stain was found to require acid hydrolysis of the cells similar to that necessary for the Feulgen reaction. Following hydrolysis, the cells were mordanted in 2 per cent formalin for two to four minutes, washed and then stained ten minutes in 0.04 normal hydrochloric acid which contained basic fuchsin in a concentration of 0.5 per cent (0.25 per cent may also be used). Destaining was accomplished in the alcohol-water mixtures during dehydration. Direct observation of the degree of destaining was made at each step of the procedure and the length of exposure of the cells to the alcohols was adjusted accordingly.

Tissues of experimentally infected mice and from a dog which had spontaneous blastomycosis were stained by the routine iron alum hematoxylin-eosin method, by the formaldehyde basic fuchsin method, and with the Feulgen stain.

Since the major portion of these observations were made, confirmatory studies using a new staining procedure which requires special methods have been made. This procedure will be reported in detail elsewhere (**5**).

CULTURAL METHODS

The yeastlike phase of *Blastomyces* was grown on dextrose nutrient agar at 37° C. For complete study of this phase, cultures

of all fourteen strains were fixed every hour for twenty-four hours. The filamentous phase was grown on cornmeal agar or Czapek's agar and on modified Sabouraud's agar at 30° C., or on cellophane membranes laid on the surface of the agar.

OBSERVATIONS

The yeastlike phase.—When grown at 37° C. on several media *Blastomyces* converts to a yeastlike growth. Levine and Ordal (**13**) have considered temperature, moisture and pH, in that order, the most important factors which operate in this conversion. To these must be added the inherent characteristics of the strain under observation. This last factor has been found to be as important as the others and is at present being studied.

The organism in the yeastlike phase grows in two forms with various intergrades. In the most common form the cells are elongated and suggest abortive hyphal elements (FIG. 3, *l* and *p*). In the second the cells are round or oval and simulate true bud-cells more closely (FIG. 5 *a*). The latter type simulates most closely that seen in tissue (FIG. 2, *A* and *B*).

Distribution of nuclei within the cells.—All cells, with rare exceptions, are multinucleated. In the majority of cells the nuclei occur as clusters or ringlike groups within cytoplasmic concentrations surrounding central vacuoles (FIG. 1, *A, B, H, L, M* and *Q*). More rarely they appear more evenly distributed throughout the cells and occur in the cytoplasmic strands separating vacuoles (FIG. 4, *a, c* and *d*). Frequently they occur in groups at the tips of cells but more often in the central part. The numbers of nuclei vary per cell. There is, however, a rough relationship between the size of the cell and the number of nuclei it contains. No nuclear counts and cell measurements have been made as yet. Nuclei have not been observed to divide into new cells or buds, but to migrate during their resting stages (FIGS. 1, *E, F* and *M* and 4*b*). There seems to be no immediate direct relationship between nuclear division and formation of new cells. When a new bud is partially formed, resting nuclei migrate into it. Budding occurs by the rupture of the outer wall and extension of the protoplast and inner wall through the aperture. The frayed edge of the outer wall is usually visible (FIG. 1, *E* and *M*).

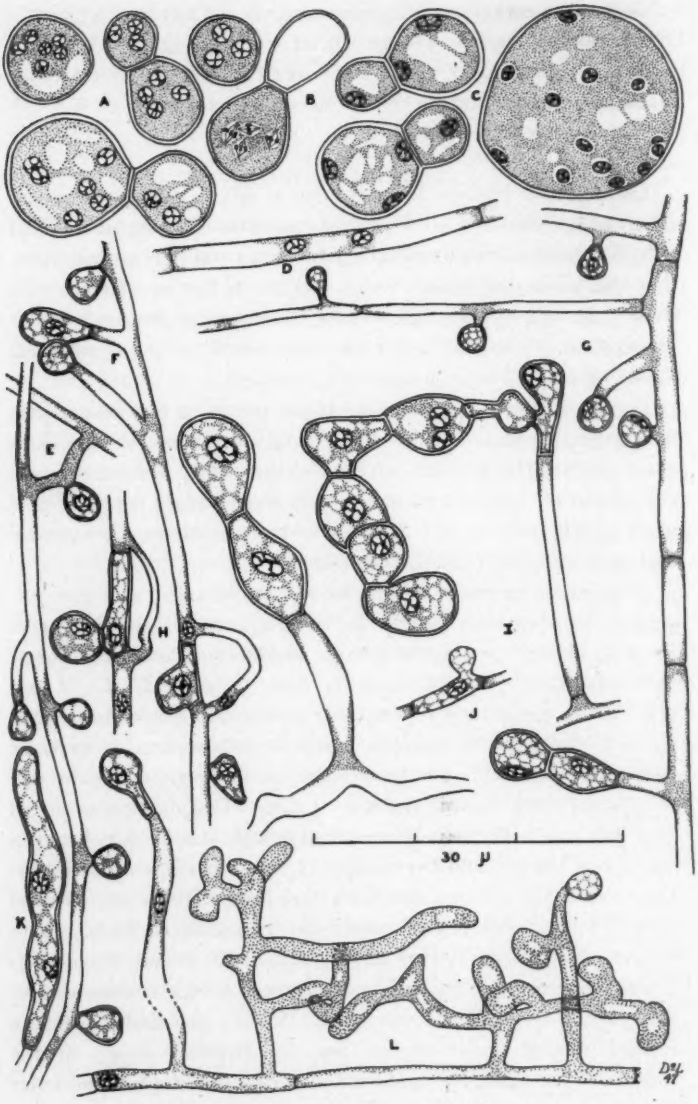

FIG. 2. *Blastomyces dermatitidis.*

The filamentous phase.—In the filamentous stage grown at 30° C. or less on a variety of media the fungus forms a typical filamentous mycelium (FIG. 2, *D* to *L*).

The cells of the filamentous phase are likewise multinucleated. There is, or appears to be, no constant positional arrangement of nuclei, except that actively growing hyphal tips contain greater numbers (FIG. 1, *E*). The number of nuclei per cell also varies within wide limits.

The septations appear to be perforated. Odd shunt tubes are to be observed when solid cross walls have been laid down (FIG. 2, *H*). Hyphal fusions are common (FIG. 2, *E*).

Conidia are produced in the filamentous phase. These vary widely in length from one to ten or more microns but have a fairly constant pyriform shape. They are relatively thick-walled. The walls are smooth. Odd angular forms, however, are also to be observed (FIG. 2, *E* to and chains of cells of a sporelike character occur (FIG. 2, *I*). The number of conidia varies markedly from strain to strain.

It has been considered by DeMonbreun (**8**) that the yeastlike phase originates from the filamentous phase by conversion through chlamydospore-like masses which reproduce. Further evidence for such a mechanism has been observed for the round cell type (**4**). The abortive hyphal element type of yeastlike growth appears, however, to be more closely related to true filamentous growth.

Sexuality.—No evidence, either morphologic or nuclear, for the occurrence of sexuality in *Blastomyces dermatitidis* has yet been observed.

The nuclear cycle.—The nuclear phenomena appear to be related strictly to the vegetative growth. Nuclear division and the formation of new cells appear to be two distinct and separate processes. Nuclear division occurs independently of budding. New cells appear to obtain their nuclei by migration of vegetative or resting nuclei into them (FIG. 1, *E*, *F* and *M*).

Following what appears to be a bona fide telephase the chromatinic granules or chromosomes form a dense mass (FIGS. 1, *O*, 3, *k* and *l*). These separate and are observed as discrete gran-

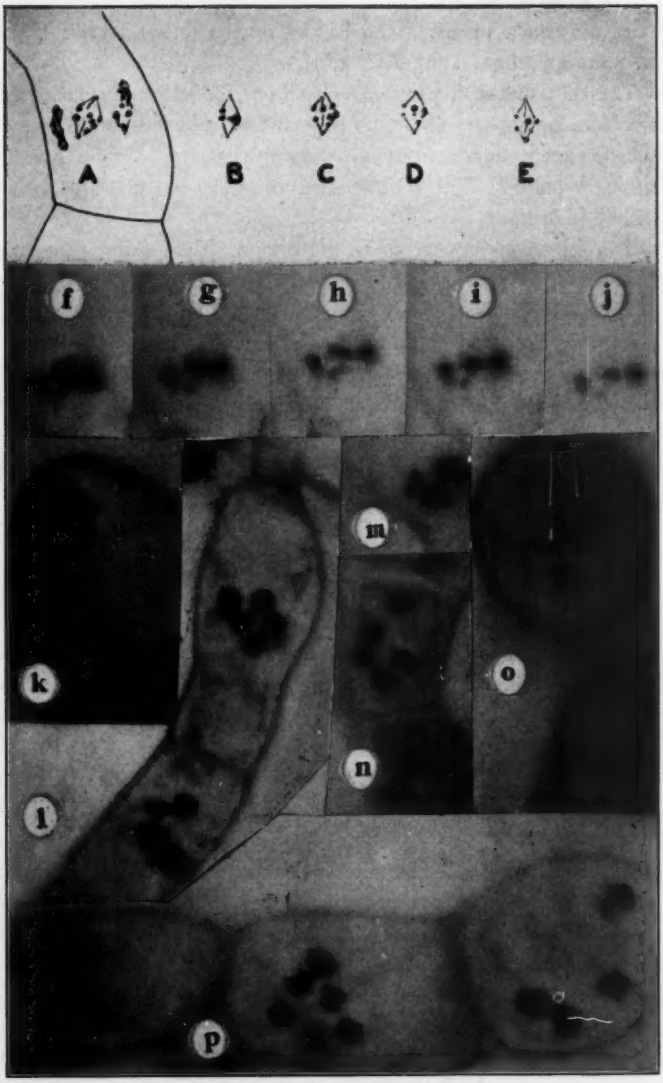

FIG. 3. *Blastomyces dermatitidis.*

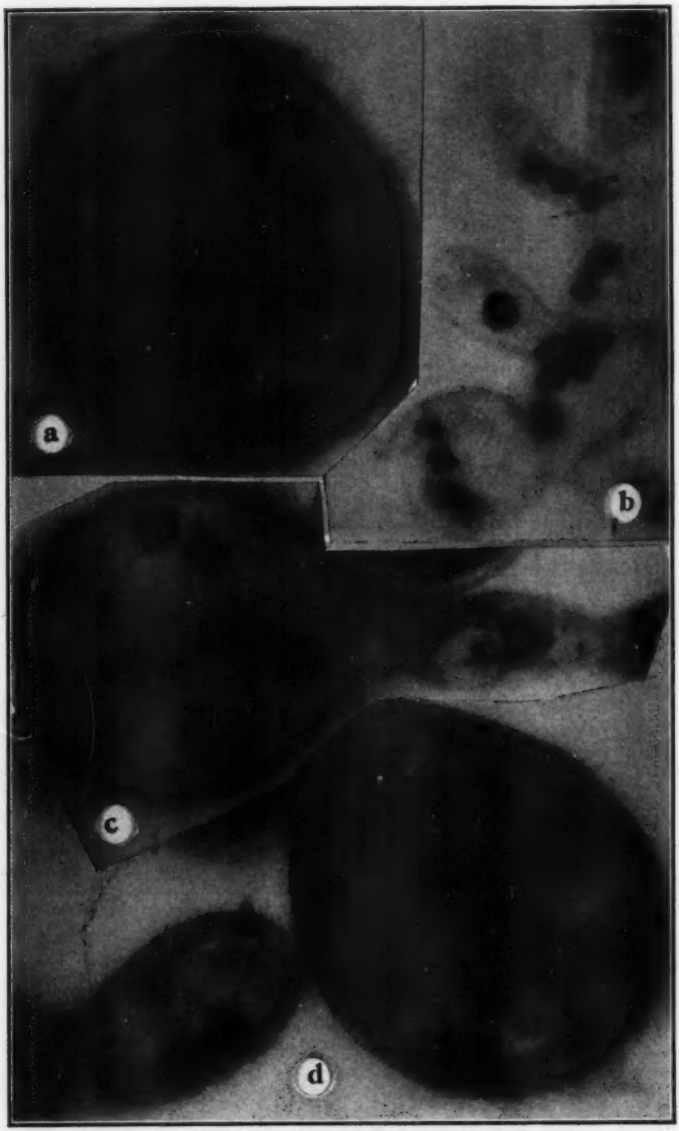

Fig. 4. *Blastomyces dermatitidis.*

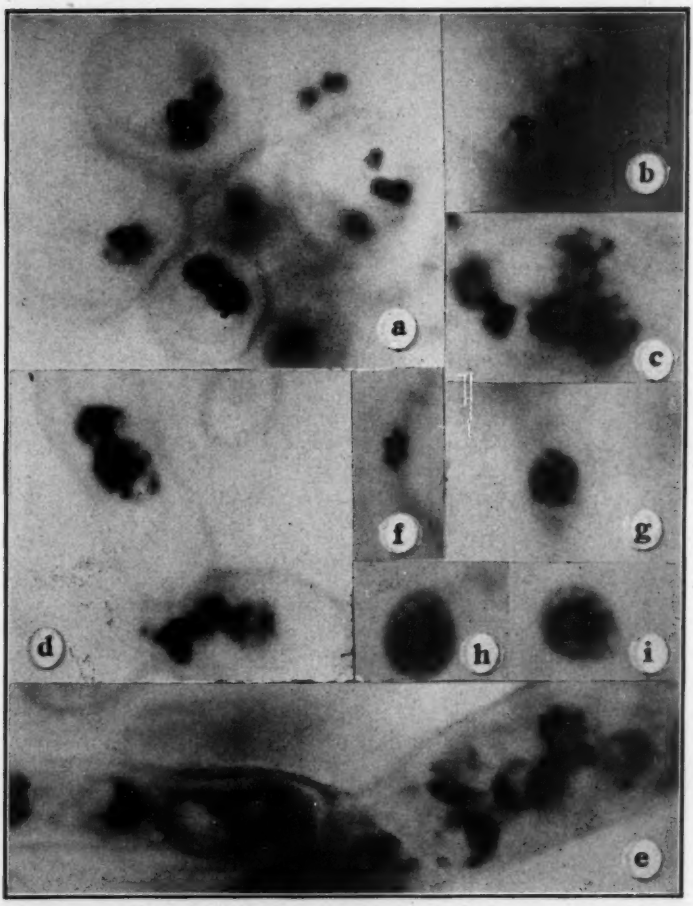

FIG. 5. *Blastomyces dermatitidis.*

ules which are integral parts of delicate threads (FIGS. 1, *P*, *Q* and *A*, and 3, *m* to *p*).

These minute resting or vegetative nuclei then undergo progressive enlargement (FIGS. 1, *A* to *F*; 3, *k* to *p*, and 5, *a* to *d*). All the nuclei within a single cell appear to be in the same stage of development, but nuclei within adjacent cells of the same cell

clump may be in distinctly different stages (figs. 4*b* and 5*a*). The granules become larger and the delicate linin net connecting them becomes more distinct. Frequently a darker mass or ringlike structure suggestive of a nucleolus is visible (fig. 5, *c* and *d*).

Observation is frequently impeded by the delicacy of the structures under observation and by the large numbers of individual nuclei present, overlapping of which causes confusion. Late resting nuclei may reach a considerable size.

The stages between what has been interpreted as early prophase and metaphase are not entirely clear. From what it has been possible to observe, however, the following sequence of events seems likely. The chromatinic threads condense and appear to shorten (figs. 1, *G* to *J*, and 5*e*). They then aggregate at one side of the nucleus and a granule appears opposite this mass (fig. 5*e*). This granule enlarges and becomes double (fig. 1, *J* and *K*). The next stage observed with assurance is the metaphase (figs. 1, *L* and *M*, and 3, *A* to *j*). It is, however, thought that these two granules separate and migrate to the opposite poles, and that the chromatinic granules (chromosomes) line up between them.

The chromosomes or chromatinic granules become double (split?) and are drawn to the two poles (figs. 1, *L*, *M* and *N*, and 4*a*), forming dense masses. The whole cycle then is repeated as described.

The difficulties encountered in making observations on such minute structures are manifold. The presence of more than one nucleus per cell is confusing.

The minute size of the chromosomes prohibits an accurate count. There appear to be four optically resolvable granules. Whether these six granules represent the actual number of chromosomes in *Blastomyces* is not certain.

SUMMARY AND CONCLUSIONS

The nuclear cytology of *Blastomyces dermatitidis* has been described. Comparable results were obtained with iron alum hematoxylin stain and with basic fuchsin stain mordanted with formaldehyde. Similar results were obtained with a new and as yet unreported staining procedure.

From the observations reported it would appear that *Blastomyces* undergoes vegetative nuclear division in a manner comparable to other organisms.

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DESCRIPTION OF FIGURES

FIG. 1. *Blastomyces dermatitidis.* Drawings made with aid of a camera lucida. Cells drawn from cultures grown at 37° C. on dextrose nutrient agar. *A.* Small bud-cell showing ten nuclei in early resting stage arranged in cytoplasmic bridgelike concentration about a central vacuole. *B.* Larger bud-cell showing nuclei in a similar cluster; nuclei are larger than in *A* and linin net pattern with chromosomes is more distinct. *C.* Abortive hyphal type of growth showing numerous vacuoles and larger resting nuclei than in *B.* Larger dark masses within nuclear matrix are interpreted as being nucleoli. *D.* Bud-cell with two large resting nuclei in which linin net

and chromosomes are obvious. *E*. Cell giving rise to abortive hyphal element. Two small resting nuclei are present in mother cell; many large resting nuclei are present in abortive hyphal cell. Branch of latter shows no nuclei. *F*. Cell developing bud into which large resting nucleus is migrating. *G*. Bud-cell showing many nuclei in same stage. Chromatin masses are beginning to aggregate and become dense. This stage is interpreted as an early prophase. *H*. Bud-cell showing nuclei about vacuole in slightly earlier stage of chromatin condensation than in *G*. *I*. Bud-cells showing characteristic double wall at attachment. The smaller cell contains two large resting nuclei. The large cell contains seven nuclei in prophase in which there appears to be a linkage of all chromatin masses to a single slightly larger granule. This figure shows that all nuclei within a single cell are in the same phase or stage of division, but that adjoining cells may have nuclei in different stages. *J* and *K*. Bud-cell and abortive hyphal element containing large nuclei in a stage interpreted as further advanced into prophase than the nuclei in the large cell in *I*. The excerpts in *K* show a lower focal level and a suggestion that the large granule to which the others are tied is dividing. Stages between this and the metaphase have been difficult to follow due to the small size and complexity produced by the presence of many nuclei. *L* and *M*. Bud-cells and abortive hyphal elements showing stages interpreted as metaphase and early telephase. The chromosomes are lined up on a plate or appear to be separating. *N*. Bud-cells containing stages interpreted as late telephase clusters of granules widely separated with suggestion of lines between. *O*. Giant round cell. Such cells are common in many strains and also occur in tissue. Numerous nuclei in stages interpreted as late telephase present. *P* and *Q*. Bud-cells and abortive hyphal elements containing stages interpreted as nuclei reorganizing into resting or vegetative nuclei. The chromosomes are dense and appear to be organizing into nuclei comparable to those in *A* at which point the cycle repeats.

Fig. 2. *Blastomyces dermatitidis* in tissue, *A* to *C*; grown on cornmeal extract agar at 30° C., *D* to *L*. *A*. Bud-cells in tissue showing resting nuclei of different size comparable to those observed in cultures grown at 37° C. *B*. Metaphase stages observed in one cell. *C*. A giant cell with many resting nuclei observed in tissue. *D*. Single cell of hypha showing two large nuclei in late resting stage in cytoplasmic condensations within hypha. *E*. Hypha bearing several pyriform conidia. The nuclei in these stained too densely to show detail. A hyphal fusion is also present from which a uninucleate conidium has arisen. *F*. Hypha showing additional conidia in one of which the nucleus could be visualized. Lower on the same filament at *H* there are intercalary conidia or chlamydospores, the walls of which apparently prevent cytoplasmic flow as tubes which are interpreted as shunts have formed, circumventing them. *G*. Additional hypha showing branching and septate condition. Conidia are uninucleate. A bicellular spore is shown arising from the lower end of an hypha. *H*. See *F*. *I*. Large uninucleate and binucleate cells in chains suggestive of aberrant conidia or chlamydospores. *J*. Thin-walled uninucleate cells interpreted as early stage in development of intercalary conidium. (See *H*.) *K*. Very long aberrant conidial or chlamydospore form containing two resting nuclei. *L*. Odd, dense,

contorted filaments, the function of which is not clear. Nuclei could not be visualized due to dense cytoplasmic staining.

FIG. 3. *Blastomyces dermatitidis* grown on dextrose nutrient agar at 37° C. (basic fuchsin stain with formalin mordant). *A*. Abortive hyphal element showing three nuclei in division. The central figure is interpreted as in metaphase in longitudinal section (× 3,500). *f*. Same as *A* at about the same focal level (× 3,400). *B*. Same as part of *A* at a high focal level showing three resolvable masses (× 3,500). *g*. Same figure as *B* at same level showing three resolvable masses (× 3,400). *C*. Same figure as *B* at a focal plane 0.25 μ lower than *B* showing several masses (× 3,500). *h*. Same figure as *C* at same level showing increasing complexity of figure (× 3,400). *D*. Same figure at a focal plane 0.25 μ lower than *C* showing decrease in size of individual bodies (× 3,500). *i*. Same figure as *D* at about the same focal level (× 3,400). *E*. Same figure at a focal plane 0.25 μ lower than *D* showing further diminution in number and mass of bodies (× 3,500). *j*. Same figure as *E* at about same level (× 3,400). *k*. Many dense early resting nuclei concentrated in tip of bud-cell (× 3,400). *l*. Localization of nuclei in central portion of cells in abortive hyphal element. Nuclei slightly larger than in *k* (× 3,400). *m*. Early resting nuclei showing breakup of dense chromatin mass into discrete chromosomes on linin net (× 3,400). *n* and *o*. Further stages of same (×3,400). *p*. Abortive hyphal element showing clumping of nuclei in central portion of cells. Nuclei have further assumed characteristic resting pattern. All nuclei in a single cell appear to be in same stage.

FIG. 4. *Blastomyces dermatitidis* in yeastlike phase grown on dextrose nutrient agar at 37° C. (basic fuchsin stain with formalin mordant). *a*. Giant cell with many nuclei. Pair in upper left hand portion are regarded as being in late telephase (× 3,400). *b*. Cluster of bud-cells and abortive hyphal elements showing multinucleate condition and nuclei in different resting stages in adjoining cells (× 3,400). *c* and *d*. Two focal levels of same giant cell showing multinucleate condition. All nuclei in same stage, interpreted as in moderately advanced resting stage. Cell in left lower corner shows torn outer wall from separation of cells (× 3,400).

FIG. 5. *Blastomyces dermatitidis* grown on dextrose nutrient agar at 37° C. and stained with basic fuchsin with formalin mordant. *a*. Cluster of budding cells showing nuclei in various resting stages from very early to late in which there are linin net and chromosomes, as well as a dark central body which may be a nucleolus (× 3,400). *b*. Medium resting stage showing linin net (× 3,400). *c*. Four large nuclei in late resting stages in which linin net is apparent and nucleolus-like body appears as a ring (× 3,400). *d*. Stage in abortive hyphal elements similar to *c* (× 3,400). *e*. Stage interpreted as early prophase in which chromosomes have aggregated to one side of nucleus opposite a faint granule (× 3,400). *f*. Distorted resting stage in hypha (grown on cornmeal extract agar at 30° C.) suggesting telephase. *g*. Late resting stage in large hyphal element, with central nucleolus. *h* and *i*. Uninucleate conidia borne on filamentous stage grown at 30° C.

SOIL PHYCOMYCETES FROM BIKINI, ENIWETOK, RONGERIK AND RONGELAP ATOLLS

F. K. SPARROW [1]

(WITH 19 FIGURES)

One of the happier aspects of the late world conflict has been the occasional opportunity given trained scientific personnel since cessation of hostilities to visit certain areas ordinarily inaccessible to them. Particularly is this true of biological work that has been done in the Marshalls. Located as they are in the mid-Pacific, it is uncertain how soon, if ever, a privately financed scientific expedition could have carefully investigated this remote group of atolls.

Rogers (**1947**) has recently given a review of the very scanty literature dealing with Marshallese fungi and notes that prior to his own collections made in 1946 through the cooperation of the U. S. Navy and the University of Hawaii, only 16 species of fungi had heretofore been reported. His first paper lists 34 species, with a promise of more to come. Of these, *Glaziella aurantiaca* was a Phycomycete belonging to the Endogonales, the others being Myxomycetes, Basidiomycetes and Fungi Imperfecti.

The present study of soil Phycomycetes from this area was made possible through the kindness of Prof. W. R. Taylor, a member of the technical staff of Joint Task Force One engaged in "Operations Crossroads." A series of 44 soil samples was obtained from Bikini, Eniwetok, Rongerik and Rongelap Atolls in the northern Marshalls. The samples were placed in small boxes and thoroughly sealed at the time of collection to prevent mixture and sent in small lots by plane to Ann Arbor. There, they were used in the preparation of gross water cultures and baited with bits of boiled

[1] Contribution from the Botany Department, University of Michigan No. 882.

grass, hemp seed, cellophane, etc., the usual method of obtaining phycomycetous fungi.

The samples were all collected at the surface of the ground and usually in thickets or coconut groves where there was some organic matter mixed with the ground and weathered coral. In those from Bikini Atoll, twelve were collected before the two blasts and eight afterwards. The post-blast samples were all from sites facing the target area. One, from Romurikku I., was taken from just above the wave line produced by the second atom bomb.

Because of the origin of these atolls and their remoteness from continental land masses, there seemed little likelihood of recovering

TABLE I

RESULTS OF SEARCH FOR PHYCOMYCETES IN SOILS FROM CERTAIN ATOLLS OF THE MARSHALL ISLANDS

Bikini			Eniwetok		Rongelap		Rongerik	
Island	Fungi Recovered		Island	Fungi Recovered	Island	Fungi Recovered	Island	Fungi Recovered
	Preblast	Postblast						
Bikini* (7)**	− − + +	− + −	Bogon (2)	+ −	Rongelap (4)	− + + −	Bock (1)	+
Namu (3)	+ −	−	Aaraanbiru (1)	+	Rigonman (1)	−	Latoback (1)	−
Enyu (4)	+ −	+ −	Aomon (2)	+ −	Busch (1)	−	Enyvertok (1)	+
Romurikku (2)	−	+	Japtan (2)	+ +	Summary			
Eniirikku (1)	+		Jieroru (1)	−	Bikini Atoll	Soil Collections	Fungi Recovered	
Chieerete (1)	−		Igurin (1)	+	Preblast Postblast	12 8	5 4	
Airukiraru (1)	−		Eleugelab (1)	+	Eniwetok Atoll	15	9	
Ourukean (1)		+	Runit (2)	+ −	Rongelap Atoll	6	2	
			Rujoru (1)	+	Rongerik Atoll	3	2	
			Giriinien (1)	−	Total	44	22	
			Bogombogo (1)	−				

* A plus sign indicates the presence of phycomycetous fungi; a minus sign, their absence.

** Number of soil samples.

from them such delicate fungi as Phycomycetes. In the preceding table are summarized the results of this search.

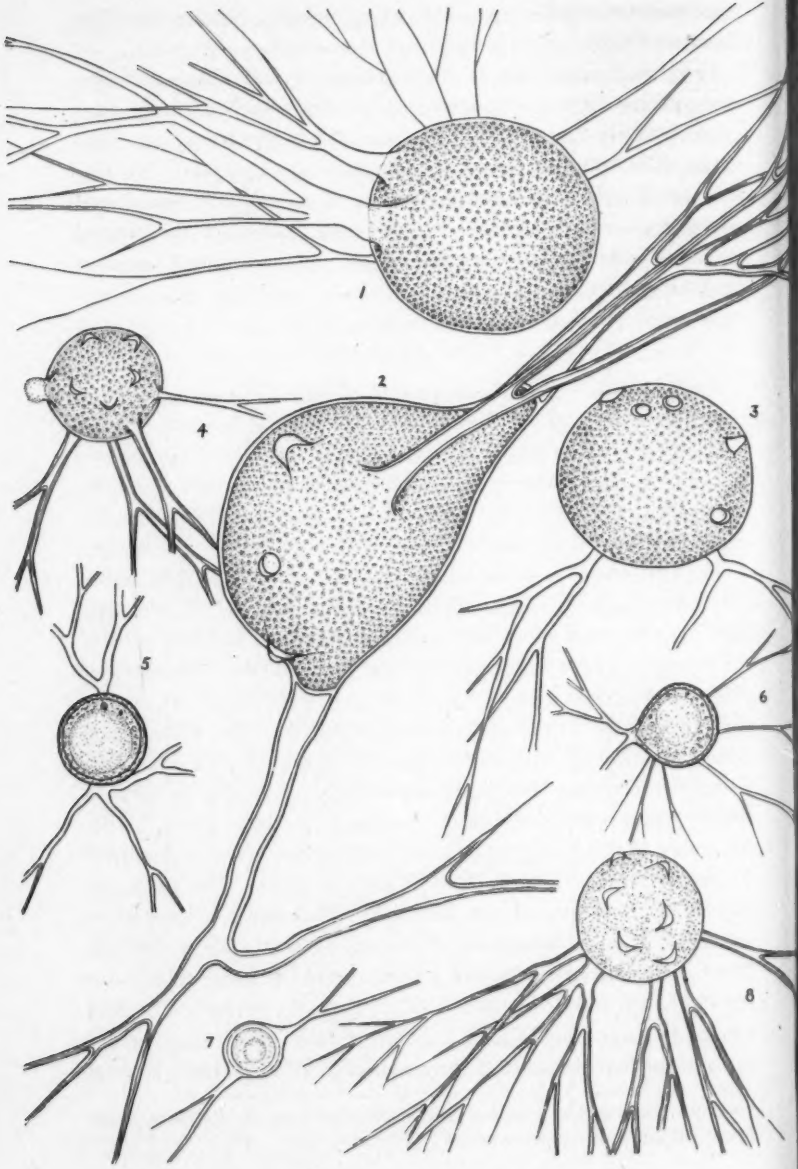
From past experience in investigating tropical soils using similar methods, it was expected that if phycomycetous fungi were present they would be such groups as the Chytridiales, Saprolegniales, Blastocladiales, Monoblepharidales and *Pythium*. No filamentous Phycomycetes, however, were recovered. Of those found in twenty-two soil samples, twenty were species of the chytrid genus *Rhizophlyctis*, the other two were chytridiaceous parasites of these species.

FUNGI RECOVERED

Rhizophlyctis spp. Three general groups of sporangial types belonging to this genus were recovered. Owing, however, to the present confused status of the species and, indeed, of our understanding of *Rhizophlyctis*, it seems better at the moment simply to characterize the groups until such time as specific and generic concepts become more clearly defined.

The two groups most commonly present both possess light amber colored mature sporangia (near "xanthine orange" [2]). In one they are somewhat spherical, 50–80 μ in diameter, and bear moderately coarse, richly branched rhizoids (FIGS. 4, 8). Members of this group occurred most frequently and were found on one or more islands of all the atolls except Rongerik. The other group forms spherical or irregularly ellipsoidal sporangia 115 μ or more in largest diameter and bears exceedingly stout (up to 25 μ in width), and extensive, much branched rhizoids (FIGS. 1–3). Members of this group were found only in soil from Japtan I. of Eniwetok Atoll and Bock I. of Rongerik Atoll. The zoospores formed by both groups are essentially alike, being ellipsoidal to spherical, 3 μ in diameter, and bearing several minute droplets. They emerge from few to many stout, short discharge tubes after the discharge and deliquescence of a gelatinous bubble. The first emerged spores usually form a coherent mass which soon breaks up, and further discharge is the result of flagellar action. Several

[2] Color terms within quotation marks are taken from R. Ridgway, Color Standards and Color Nomenclature, Washington, D. C. 1912.



FIGS. 1–8. Phycomycetes from Marshall Islands.

types of ellipsoidal or spherical resting spores have been observed associated with the amber-colored sporangia (FIGS. 5, 6). The spherical ones are 30–35 μ in diameter, the ellipsoidal 35 × 40 μ. Both have a thickened, nearly smooth brownish wall and brownish contents within which are a large oil globule and several smaller ones.

The members of these two groups closely approximate plants which have been ascribed to *Rhizophlyctis* (*Karlingia*) *rosea* by Ward (**1939**), Johanson (**1944**) and Karling (**1947**) and to *Entophlyctis aurea* by Haskins (**1946**). The coloration, however, is certainly not "pink." It should be noted that the color of the cytoplasm of *R. rosea* has been stated by Karling (**1947**) to vary from rosy pink or light orange to golden.

Color is emphasized here because of its bearing on the third of the three groups of species found. Members of this group occurred intermixed with the amber-colored forms and were recovered from Ourukeen I., Bikini Atoll, Igurin I., Eniwetok Atoll and Rongelap I., Rongelap Atoll. Several well marked features distinguished them. Although the sporangia were occasionally spherical (FIG. 9) more often they were irregularly angular due primarily to the strongly flaring bases of the broad discharge tubes (FIGS. 10, 11). The rhizoids were never so broad as in the amber-colored forms and, most striking of all, the contents were shot through with red granules (near "Carmine Red" and "Nopal Red"). The zoospores were colorless and little different in size and shape from those previously described. The several granules in them were exceedingly minute.

Olpidium Rhizophlyctidis n. sp.

Sporangia subglobosa vel ellipsoidea, 25–50 μ longa, diametro 12–45 μ, hyalina, leviter circumvallata, tubo latiusculo singulo praedita ad exteriorem; zoosporis numerosis, globosis, ca. 2 μ diametientibus, absque, globulo conspicuo, sed flagellum longum posteriorem ferentibus, erratice motilibus motu interdum saltante interdum natante; sporis perdurantibus sphaericis, 12–20 μ diametro, pallide brunneis, ut videtur asexualiter formatis, pariete laevi ca. 2 μ crassa, uniguttulata. Germinatio mihi ignota.

Parasiticum in thallis sporangiisque generis *Rhizophlyctidis* ad insulas Bikini, Eniwetok et Rongelap, in Insulis Marshallianis.

Sporangia spherical or ellipsoidal, 25–50 μ long by 12–45 μ in diameter, hyaline, smooth-walled, with a single discharge tube

which protrudes through the host wall; zoospores numerous, spherical, about 2 μ in diameter, without a conspicuous globule, posteriorly uniflagellate, movement an erratic hopping interspersed with periods of swimming; resting spore spherical, 12–20 μ in diameter, faintly brown, apparently asexually formed, with a smooth wall about 2 μ thick, contents with a large fat droplet, germination not observed.

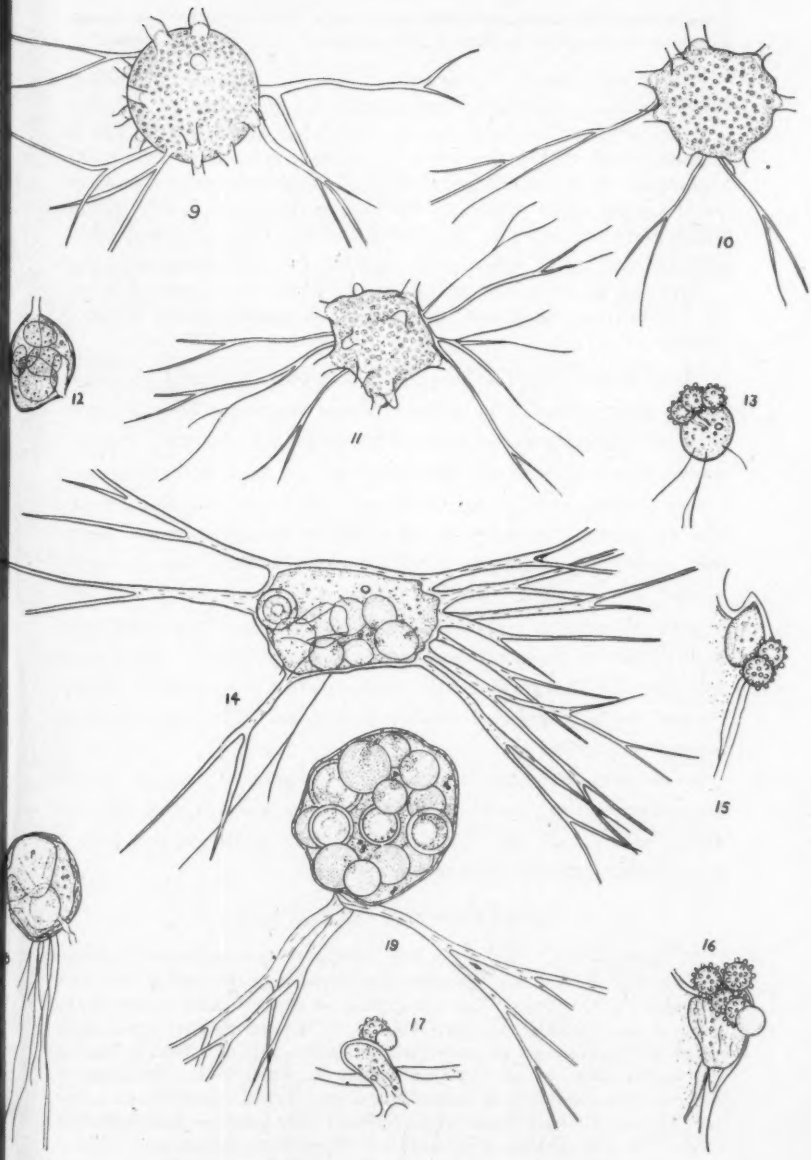
Parasitic in thalli, sporangia and resting spores of *Rhizophlyctis* spp., Bikini, Eniwetok and Rongelap atolls, Marshall Islands.

In the thalli, sporangia and resting spores of the amber colored types of *Rhizophlyctis* from Bikini I., Enyu I., and Eniiruku I. of Bikini Atoll, Bogon I. of Eniwetok Atoll and Rongelap I. of Rongelap Atoll, there were found thalli of the endobiotic parasite (FIGS. 7, 8, 12, 14, 18, 19) described above. One to many spherical or ellipsoidal hyaline thalli 12–45 × 25–50 μ were present in a single host plant. At maturity some of the parasitic thalli became converted into sporangia, each provided with a single slender discharge tube which penetrated the wall of the host and through which the zoospores were discharged. The zoospores were spherical, about 2 μ in diameter, posteriorly uniflagellate and without the usual distinctive globule. Their movement was of a rapid and erratic hopping type. Other thalli became converted, apparently asexually, into resting spores (FIG. 7, 19). These were spherical, faintly brown, 12–20 μ in diameter with a smooth wall about 2 μ in thickness. As many as sixteen have been found in a single host thallus. Their germination was not observed.

So far as the writer is aware, this is the first undoubted species of *Olpidium* parasitic on another Phycomycete and, with *O. Uredinis,* one of the only two species known to attack fungi. Like other members of the genus, it has little to distinguish it morphologically and is segregated on the basis of its host plant.

Rhizophidium marshallense n. sp.

Sporangium sessile, globosum, 10–12 μ diametro, hyalinum, leviter circumvallata; parte endobiotica ex rhizoideo tenui 1-ramoso constante; zoosporis subovoideis, ca. 2 μ diametro, globulo singulo minuto refractivo et flagello posteriori praeditis, per poro singulo varie locato ad exteriorem natantibus; sporis perdurantibus globosis, 10–15 μ diametro, pallide aureis, dense bullationibus prominentibus vestitis; parte endobiotica eae sporangii simili. Germinatio ignota.



FIGS. 9–19. Phycomycetes from Marshall Islands.

Parasiticum in thallis sporangiisque generis *Rhizophlyctidis* ad insulas Eniwetok et Rongelap, in Insulis Marshallianis.

Sporangium sessile, spherical, 10–12 μ in diameter, colorless, smooth-walled; endobiotic part consisting of a slender, at least once-branched, rhizoid; zoospores somewhat ovoid, 2 μ or less in diameter, each with a single hyaline, minute refractive globule and a posterior flagellum, escaping through a minute variously placed pore; resting spore spherical, 10–15 μ in diameter, faintly golden and densely covered with prominent knoblike bullations, endobiotic part like that of the sporangium; germination not observed.

Parasitic on thalli and sporangia of *Rhizophlyctis* spp., Eleugelab I. Einwetok Atoll and Rongelap I., Rongelap Atoll, Marshall Islands.

This species of chytrid was found attacking thalli and sporangia of the rose colored and amber colored forms of *Rhizophlyctis*. Mature thalli consisted of a spherical, 10–12 μ in diameter, smooth-walled, colorless, epibiotic part sessile on the wall of the host, and a very slender, at least once-branched, endobiotic rhizoidal system. The zoospores formed by the sporangia were 2 μ or less in diameter, posteriorly uniflagellate, and escaped through one variously located, minute pore. Resting spores were produced quickly and in great abundance. They were apparently asexually formed from thalli similar to those which gave rise to zoosporangia. They were spherical, 10–15 μ in diameter, faintly golden colored and densely covered with prominent hyaline bullations. Their germination was not observed.

A comparison of this chytrid with congeneric parasitic forms possessing resting spores with ornamented walls reveals that by reason of its small size, spherical uniporous sporangia and host it is a distinct, hitherto undescribed species.

EXPLANATION OF FIGURES

All figures × 330. FIGS. 1–3. Large amber colored sporangia of *Rhizophlyctis* spp. FIGS. 4, 8. Sporangia characteristic of the smaller size class of amber colored forms. The sporangium of figure 8 bears within it the thalli of an endobiotic parasite. FIGS. 5, 6. Brown resting spores associated with populations of amber colored forms. FIG. 7. Thallus bearing the resting spore of an *Olpidium* within it. FIGS. 9–11. Sporangia of *Rhizophlyctis* illustrative of populations of pink to red colored forms. FIG. 12. Thallus of *Rhizophlyctis* heavily infected with *Olpidium Rhizophlyctidis* n. sp. FIG. 13. Thallus of *Rhizophlyctis* bearing resting spores of *Rhizo-*

phidium marshallense n. sp. FIG. 14. Peltate thallus of *Rhizophlyctis* with thalli, sporangia and one resting spore of *Olpidium Rhizophlyctidis*. FIGS. 15–17. Sporangia and resting spores of *Rhizophidium marshallense* on thalli of red pigmented forms of *Rhizophlyctis*. FIGS. 18, 19. Thalli, empty sporangia and resting spores of *Olpidium Rhizophlyctidis* on amber colored forms of *Rhizophlyctis*.

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NOTES ON THE GENUS CYSTODERMA

ALEXANDER H. SMITH AND ROLF SINGER

(WITH 1 FIGURE)

Since publishing our monograph on the genus *Cystoderma*[1] further information worthy of note has accumulated. A new species with very peculiar characters was discovered on the slopes of Mt. Hood, Oregon. A second undescribed species was found south of Mt. Hood, and a number of other interesting collections have been made in the Mt. Hood National Forest.

SUBGENUS **Dissoderma** subg. nov.

A subgenere **Eu-Cystoderma** subgen. nov. (= *Cystoderma* in limitibus Monographiae auctorum) differt epithelio summopere heterogeneo, in pileo evolutione in juvenilibus terminato et expansione pilei dissociato delapsoque ita ut pilei maturi epicute careant; sporis inamyloideis, majusculis.

Cystoderma paradoxum sp. nov. (FIG. 1).

Pileo 1–3 cm. lato, convexo, appresse fibrilloso, pallide lilaceo vel obscure violaceo-livido, pallidiore in speciminibus siccioribus, in primordiis velo granuloso ferrugineo celluloso obtecto sed dein denudato tegumenti separatione a superficie pilei. Lamellis distantibus vel subdistantibus, latis, arcuato-adnatis vel subdecurrentibus, interdum postremum adnexis et dente decurrentibus et tunc frequenter ventricosis in maturis, pallide brunneolo-lividis vel pallidissime opace purpurascentibus, aciebus integris. Stipite 4–8 cm. longo, 4–6 mm. lato in tertia apicali, 6–8 mm. deorsum, pallide lilaceo lacero-squamoso longitudinaliterque striato in tertia apicali, solido, in duobus tertiis inferioribus cothurnato tegumento velari granuloso, cinnamomeo-alutaceo vel argillaceo-brunneolo quo rupto armillis granulosis concentricis vel fasciculis vel squamis obtectae apparent. Carne grisello-violacea, violacea vel lilacea in pileo et in tertia apicali stipitis, argillaceo-brunneola in parte inferiore stipitis; odore saporeque haud notabilibus. Sporis plerumque 9–10 × 4.5–6 μ, ellipsoideis, laevibus, membrana simplici, continua, hyalina vel subhyalina (in KOH), subpseudoamyloideis, haud amyloideis; basidiis 30–35 × 7.5–10 μ; cystidiis nullis; tramate hyalino; cuticula pilei (primordiis exceptis) reducta, ex zona densiuscula et pigmentata consistente, hyphis filamen-

[1] A monograph on the genus *Cystoderma*. Papers Mich. Acad. Sci. Arts and Letters **30**: 71–124. 1945.

tosis efformata; fibulis numerosis; tegumento stipitis granuloso e catenulis cellularum ovoidearum, ellipsoidearum vel globosarum (flavido-brunnearum vel ferrugineo-vinacearum in KOH) consistente.

Ad humum et inter Bryophyta, solitarius vel e massa carnosa basali ecrescens. Octobri. Legit A. H. Smith in Mt. Hood National Forest, Oregon, Americae Borealis. **Typus** (n. S–27987) in Herbario Universitatis Michiganensis conservatus est.

Pileus 1–3 cm. broad, convex to broadly convex, sometimes with a slightly depressed disc and sometimes with a low broad umbo,

FIG. 1. *Cystoderma paradoxum.*

surface dry and appressed fibrillose, sometimes the fibrils aggregated into fascicles near the margin, matted down like felt over the disc, color pallid lilac to dark violaceous drab ("dusky drab" to "dusky brown" on disc, margin "pale brownish drab") in wet caps, paler lilac when not water soaked, some when badly water soaked translucent-striate on margin and appearing atomate when dried out at room temperature; buttons covered by a "Sudan brown" or duller granulose veil which is only loosely connected to tissue of pileus, the granules (sphaerocysts) resembling those found in *C. amianthinum* f. *typicum,* granulose coating separating from

pileus surface and sloughing off in early stages of pileus expansion thus exposing the lilaceous fibrillose cuticle as observed on mature caps; flesh thin, soft, watery, "pale brownish drab" to grayish violaceous, odor and taste not distinctive; lamellae distant to subdistant, broad, variable in manner of attachment, arcuate-adnate to subdecurrent or in some bluntly adnate becoming adnexed, with a slight tooth and this type sometimes becoming ventricose, "pallid brownish drab" to "pale ecru drab" (very pale dull purplish), edges even; stipe 4–8 cm. long, 5–6 mm. in apical third, 6–8 mm. in lower two thirds, upper third pale lilac or violaceous, and lacerate scaly as well as longitudinally striate, lower two thirds sheathed by a granulose "cinnamon buff" to "clay color" veil which is broken up into more or less concentric rings of tufts or scales, flesh within clay color in lower two thirds, lilac to violaceous in upper third, solid.

Spores (8.5)9–10(11) × 4.5–6 μ, ellipsoid, smooth, the wall thin to very slightly thickened, simple, hyaline or subhyaline in KOH and NH_4OH, yellowish to pale tawny (hence slightly pseudoamyloid) in iodine (most coloration obtained when plunged in alcohol, then in water, and finally stained with Melzer's reagent; least coloration obtained with ammonia followed by Melzer's reagent); basidia four-spored, 30–35 × 7.5–10 μ, clavate, hyaline in KOH; pleurocystidia and cheilocystidia not differentiated; gill trama hyaline in KOH, its hyphae interwoven—subparallel (not distinctly irregular); pileus trama hyaline in KOH or near surface faintly brownish gray; cuticle differentiated only as a slightly denser and pigmented zone, its elements of various shapes and without orderly arrangement, filamentous elements predominantly repent, thickened elements predominantly vertically arranged, both kinds often interwoven and with walls only slightly thicker than in trama proper; clamp connections present; covering of stipe (lower two-thirds) consisting of chains of oval, ellipsoid or globose cells with yellowish brown to rusty vinaceous walls in KOH, the walls slightly thickened, some cells rather irregular in outline or with one or two papillae; cells on surface of buttons rusty to cinnamon in KOH, mostly globose to short ellipsoid.

Habit, habitat, and distribution. Solitary or in small clusters from a fleshy basal mass of tissue. On humus and among mosses. Mt. Hood National Forest, Oregon. The following collections have been made: S–24843, Oct. 21, 1946; S–25003, Oct. 26, 1946; S–27824, Oct. 16, 1947; S–27987, Oct. 19, 1947; and S–28341, Oct. 27, 1947. The specimens illustrated, S–27987, are designated the **type**.

Discussion. We withheld publication of this species until we could ascertain the type of cuticle present in button stages. Small buttons were collected in S–27824 and the cuticle found to be similar to that of the typical form of *C. amianthinum.* The peculiar features of this fungus are many. In the first place the manner in which the cellular covering is sloughed off, so completely that no remains are left on the cap, is a sufficiently important character to justify erecting a subgenus for the species. The peculiar color of the pileus, gills and upper third of the stipe is very distinctive as a field character.

There are still some questions to be answered in regard to this fungus. One is whether or not it really grows from very decayed mushrooms or is truly terrestrial. S–25003 gave some evidence of having come from a decayed fruiting body of a fleshy fungus but the remains were too far gone for any identification. No evidence was found during 1947 to suggest such a substratum, but again, all collections had either matured fruiting bodies or buttons which had ceased developing. Stages in the development of the buttons are greatly desired in order to learn more about the manner of disjunction of the covering layer of the pileus.

Cystoderma subpurpureum sp. nov.

Pileo 4–8 mm. lato, convexo, expanso vel plano, sicco pulverulentoque atque obscure livido-brunneo, maturando pallide grisello-vinaceo, demum subdiscolorato. Lamellis late adnatis, confertis vel subdistantibus, latis, dilute rubido-vinaceis vel brunneolo-vinaceis, aciebus integris. Stipite 1–2.5 cm. longo, usque ad 1 mm. lato, pileo concolori, exsiccando purpurascente, fragmentis sparsis veli pulverulenti obtecto, zona apicali fibrillosa aut annulo mox evanescente instructo, apice pruinoso. Carne pilei vinacea, tenui; odore nullo; sapore miti. Sporis 4–4.5 × 2.5–2.8 μ, ovoideis, laevibus, hyalinis, inamyloideis; basidiis 18–20 × 4.5–5 μ; cystidiis nullis; tramate hymenophorali in juvenilibus subviolaceo-grisello (in KOH), dein subhyalino; tramate pilei subviolaceo-griseo vel hyalino in KOH; epithelio pilei primum obscure violaceo-livido in KOH, gradatim autem depallentibus usque ad sordide brunneolo-violaceum; fibulis praesentibus.

Ad locum deustum Mt. Hood National Forest, Oregon Americae Borealis Septembri 1947. Legit A. H. Smith (n. S–26824), **typus,** in Herbario Universitatis Michiganensis conservatus est.

Pileus 4–8 mm. broad, convex, expanding to plane or the margin arched and disc slightly depressed; surface dry and powdery at first, "deep livid brown" young, "pale grayish vinaceous" near ma-

turity, in age when faded ashy with a purple-drab cast (dark haematite color to pale ashy pink and fading to ashy in age); flesh thin, tinged vinaceous, odor and taste none, lamellae broadly adnate, close to subdistant, broad, "light russet-vinaceous" to "deep brownish vinaceous" (pale haematite color), edges even; stipe 1–2.5 cm. long, 1 mm. or less thick, concolorous with pileus and drying more purplish, with scattered remains of the thin powdery veil and with a fibrillose apical zone or annulus which is soon evanescent, apex pruinose.

Spores 4–4.5 × 2.5–2.8 μ, ovoid, smooth, hyaline, nonamyloid; basidia four-spored, 18–20 × 4.5–5 μ; pleurocystidia and cheilocystidia none; gill trama in young caps with a violaceous gray cast in KOH, in mature caps nearly hyaline, parallel becoming subinterwoven; pileus trama violaceous gray to hyaline in KOH; cuticle of globose to short-ellipsoid cells, or a few scattered fusoid cells also present, dark violaceous drab at first when mounted in KOH, gradually clearing to a dull brownish violaceous, the cell walls smooth and slightly thickened; clamp connections present.

Habit, habitat, and distribution. Scattered over an area where a brush pile had been burned, Clear Creek at Skyline Rd., Mt. Hood National Forest, Oregon. September 25, 1947 (S–26824 **type**).

Discussion. This fungus differs from the accounts of *C. haematites* by English authors in the much darker gills even if one wishes to disregard the striking difference in size. *C. haematites,* according to Kühner and Maire,[2] has amyloid spores, and this, of course, places the two species in different sections. In our system of classification *C. subpurpureum* would be in our section Granulosa. It differs from *C. granulosum* var. *occidentale* in the KOH reaction of the sphaerocysts as well as in its darker colors and slender stature.

CYSTODERMA GRUBERIANUM Smith[3]

This interesting species with large (9–11.5 × 5–6 μ), subfusiform, amyloid spores belonging in the *C. amianthinum* series

[2] Étude de la réaction de la membrane sporique à l'iode dans les divers genres d'Agarics leucosporés. Bull. Soc. Myc. Fr. **50**: 9–24. 1934.

[3] Mushrooms in their natural habitats illustrated with stereokodachromes. Sawyer's Inc., Portland, Oregon. (In press.)

should be called to the attention of those interested in this genus. It is a lignicolous species inhabiting rotting Douglas fir logs. A second collection (S–28239) has been obtained from the type locality. In it the spores measure up to 12.5 μ long and are strongly amyloid.

SUPPLEMENTARY NOTES ON KNOWN SPECIES

C. GRANULOSUM (Batsch ex Fr.) Fayod var. TYPICUM f. TYPICUM. Material from Errol, N. H. (Linder & Rusden, det. Singer Sept. 17, 1945, FH) and Hamilton, old Harvard Forest, Mass. (Singer, Oct. 24, 1945, FH) shows that the type form is not rare in New England.

C. GRANULOSUM var. ADNATIFOLIUM (Peck) Smith & Sing. Material from Mt. Monadnock, N. H. (Linder & Rusden, det. Singer, Sept. 20, 1945, FH) is the first material studied by one of us coming from a New England locality. Although this extension may have been expected, as the variety was known from both New York and Eastern Canada, we think it worthy of record.

C. CINNABARINUM (A. & S. ex Secr.) Fayod. A collection from Harvard, Mass. (Singer & Dadmun, September 1944, FH) is the first indubitably correct indication from Massachusetts. More important is a specimen received by the Farlow Herbarium in exchange from France (Lacorube, région Lyonnaise, coll. Locquin, no. 160.44, October 16, 1944). It has very distinct cystidia with crystals at the tip, and is entirely identical with our American material as well as with the junior author's Siberian material. Locquin is a French specialist of *Lepiota* sensu lato and it is significant that his determination (which reads: *Lepiota cinnabarina*, sensu Kühner, Locquin) fully coincides with ours.

C. CARCHARIAS f. ALBUM (Fr.) Smith & Sing. This form, previously reported for North America only from one collection in Michigan, was found near Rhododendron, Oregon, Oct. 27 (S–28882), and near Sandy, Oregon, Nov. 6, 1947 (S–28527). Collection S–28332 was from a moss carpet under second growth Douglas fir, and S–28527 was from a very dense growth of Rubus.

alder and maple, the typical "jungle" growth of the Humid Transition zone.

C. AMIANTHINUM var. SUBLONGISPORUM Sing. apud Smith & Sing. A collection from Virginia (Singer, White Top Mountain, no. V193, summer 1946, FH) extends the area of this variety to Virginia.

THE DISTRIBUTION OF SOIL MICRO-ORGANISMS ANTAGONISTIC TO FUNGI PATHOGENIC FOR MAN *

ALBERT SCHATZ † AND ELIZABETH L. HAZEN

(WITH 3 FIGURES)

Although the majority of investigations on antagonistic relationships among microorganisms have been primarily concerned with inhibition of bacteria, there are numerous reports on the antifungal properties of various microorganisms and cell-free preparations obtained from them. No attempt will be made to discuss this extensive literature reviewed so well by Waksman (**1**). These studies dealt chiefly with the antagonism of saprophytic or plant-pathogenic fungi and the fungistatic or fungicidal properties of more or less nonspecific substances produced by active cultures. Only a few (**2–10**) are comparable in scope to surveys on the distribution of microorganisms antagonistic to bacteria (**11–15**). No surveys conducted to investigate microorganisms antagonistic to fungi pathogenic for man have to our knowledge been reported.

A variety of fungi pathogenic for man, such as *Trichophyton schoenleini, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Microsporum gypseum, Trichophyton gypseum* and *Trichophyton mentagrophytes,* as well as plant pathogens and nonparasitic fungi, have been tested for susceptibility *in vitro* to different antibiotic substances. The list of agents includes actinomycin (**16, 17**), chaetomin (**17**), clavacin (**16, 17, 18**), eumycin (**19**), fumigacin (**16, 17**), gladiolic acid (**20**), gliotoxin (**16, 17, 21, 22**), glutinosin (**23**), hemipyocyanine (**24**), mycophenolic acid (**25**), penicillin

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(16, 26, 27), pyocyanase (24), simplexin (28), streptomycin (17, 29), streptothricin (17), tyrothricin (24), viridin (30), and others.

The majority of these substances were found to be highly inhibitive to fungi; eumycin, gladiolic acid, glutinosin, and viridin being appreciably more active against fungi than bacteria. On the other hand, certain antibiotics such as chaetomin, penicillin, and streptomycin exerted little or no effect on the microorganisms tested. Unfortunately, the preparations that appeared most promising *in vitro* have no widespread application because of toxicity or other undesirable pharmacologic properties. Consequently, no antibiotic agent approaching the efficacy of penicillin and streptomycin against bacterial infections is available in fungus infections either of the superficial or the deep-seated type. For this reason, experiments were undertaken to investigate (1) the distribution of soil microorganisms antagonistic to fungi pathogenic for man and (2) the antifungal agents produced by these microorganisms. This report presents chiefly the results of the study of the distribution of soil microorganisms antagonistic to certain pathogenic fungi employed as test microorganisms.

MATERIALS AND METHODS

Several different substrates were chosen as sources of microflora. Almost all the studies, however, were made with a deciduous forest soil, a field soil, and a 2 to 3-year-old leaf compost, the pH values of which were approximately 5.3, 8.4, and 6.1, respectively. A peat and a garden soil both at pH 4.5 were considered unsatisfactory and discarded after preliminary experiments because of the generally low microbial population and the almost complete absence of actinomycetes.

Three tap water media used in previous studies for the isolation of microorganisms antagonistic to bacteriophages (31, 32) were employed: nutrient broth (NaCl 0.5 per cent, peptone 0.5 per cent, meat extract 0.3 per cent); glucose-tryptone broth (glucose 1 per cent, tryptone 0.5 per cent, K_2HPO_4 0.2 per cent, NaCl 0.2 per cent, $FeSO_4$ 0.001 per cent); glycerol-yeast extract broth (glycerol 3.15 per cent, yeast extract 0.3 per cent, NaCl 0.2 per cent, K_2HPO_4

0.1 per cent, $MgSO_4$ 0.02 per cent, $FeSO_4$ 0.001 per cent). For liquid cultures of the actinomycetes, 0.15 per cent agar was added to the broths to obtain sufficient viscosity for supporting pellicles, and for the solid media, 1.5 per cent agar was added. All the media were adjusted to pH 6.8–7.2. A neutral tap water dextrose-asparagin agar (dextrose 1 per cent, asparagin 0.05 per cent, K_2HPO_4 0.05 per cent, agar 1.5 per cent) was also employed for some of the studies. The media containing glucose were autoclaved at eight pounds pressure for thirty-five minutes; the others were sterilized at fifteen pounds pressure for twenty minutes.

The pathogenic fungi employed as test microorganisms were *Candida albicans* (*No. 4657*), *Cryptococcus neoformans* (*No. 45215*), *Trichophyton gypseum* (*No. 45141*), and *Trichophyton rubrum* (*purpureum*) (*No. 4516*). The cultures were maintained on slants of glucose-tryptone agar and suspensions were prepared by adding 5 ml. of sterile saline or broth to an agar slant and scraping the growth from the surface with a stiff wire loop.

The soil microorganisms were isolated at random and also by selective plating technics. One method employed was a modification of Foster and Woodruff's procedure (**33**). The soils and compost were plated in agar seeded with a slow-growing fungus. After three to four days, the colonies of the soil microorganisms that had developed rapidly and produced diffusible antifungal substances were surrounded by clear zones in which the pathogen had been inhibited. From the clear zones the antagonists were transferred to agar slants. In addition, the soils and compost were plated and incubated for from five to six days. A second layer of fungus-seeded glucose-tryptone agar was then poured over the surface of those plates with well-isolated colonies, care being taken to prevent bacterial colonies from flowing and contaminating the entire surface. After from one to four days of further incubation, antagonistic microorganisms in the original layer of agar that were surrounded by clear zones of inhibition of the pathogenic fungus in the second agar layer were removed.

The procedures for the agar-streak, agar-dilution, and agar-cup or diffusion tests employed were essentially the same as those previously described for studies with fungi (**17**). The three agar media were used for the agar-streak tests, and glucose-tryptone

agar was used throughout for the dilution and diffusion assays. All incubation was at approximately 28° C., the duration varying with the particular experiment and the rate of growth of the test microorganisms.

EXPERIMENTAL

It was assumed *a priori* that the actinomycetes would constitute the most fruitful group among which to search for antifungal properties. The better known antibiotics of bacterial origin, such as tyrocidine, tyrothricin, subtilin, bacillin, bacitracin, simplexin, are more or less similar in chemical nature and antibacterial activity which is largely against Gram-positive bacteria. The agents produced by fungi and actinomycetes, on the other hand, appear to represent more heterogeneous types of compounds chemically, pharmacologically, and antibiotically. In numerous agar platings of mixed microbial populations from such natural substrates as soils, manures, and composts, the antagonism of fungi by actinomycetes rather than by molds has been more frequently observed. This phenomenon is illustrated by figure 1 in which all seven colonies antagonizing the spreading fungus are actinomycetes. For these reasons, attention was directed to the potentialities of the actinomycetes.

Two hundred and forty-three actinomycetes, one hundred and ninety-eight isolated at random from a leaf compost, a peat, and five soils, and forty-five previously reported to be antagonistic to bacteriophages (**32**) were tested on the three agar media by the streak method against the four pathogenic fungi. The actinomycetes were incubated for two days before being cross-streaked with the four pathogens. Because of poor growth, *Cryptococcus neoformans* was not tested on the nutrient agar. As illustrated in figure *2*, antagonistic actinomycetes exhibited zones of inhibition from two to three days after the fungi were inoculated.

Those actinomycetes that were appreciably active by the agar-streak procedure were grown in liquid media, 100 ml. per 500-ml.-Erlenmeyer flasks. After incubation for ten days, the cultures were filtered through sterile paper. Untreated samples of the filtrates were assayed by the agar-diffusion or cup technic against

Candida albicans and *Cryptococcus neoformans*. Because of the rapid growth of the former, zones of inhibition were measured after approximately eight hours. The plates with *Cryptococcus neoformans*, however, were incubated overnight (20–24 hours) before being read. Portions of a large number of the filtrates were heated

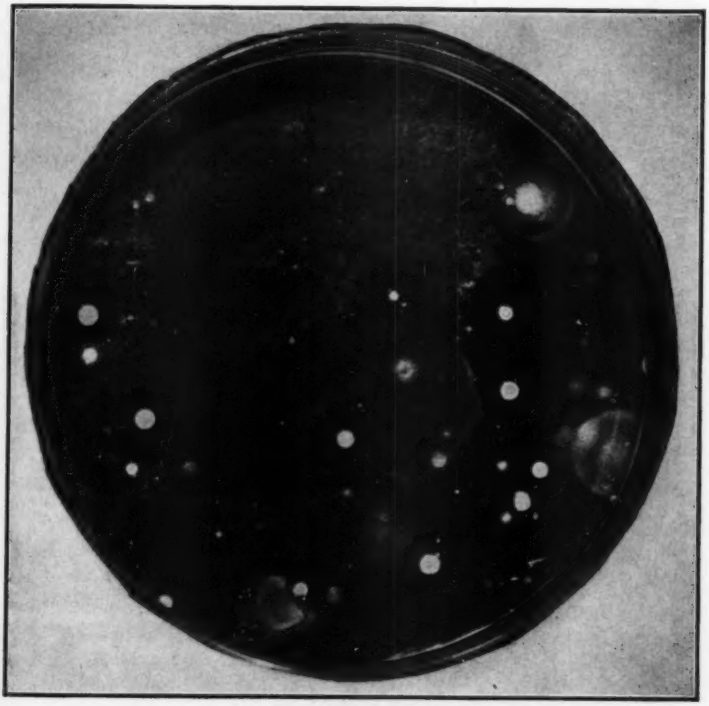

FIG. 1. Antagonism among soil microorganisms. The inhibition of a spreading fungus by actinomycetes.

at 70–75° C. for ten minutes and tested by the agar-dilution method against all four pathogens.

Table I shows that one hundred and twenty-four (51 per cent) of the two hundred and forty-three actinomycetes tested by the agar-streak method were active against one or more of the test microorganisms on at least one of the three media employed. This

value is of the same order of magnitude as the percentage of actinomycetes reported to be antagonistic to bacteria, namely 59 per cent by Nakhimovskaia (**14**) and 43 per cent by Waksman *et al.* (**11**). When the heated culture filtrates were tested by the agar-dilution and diffusion procedures, however, only 10 per cent and

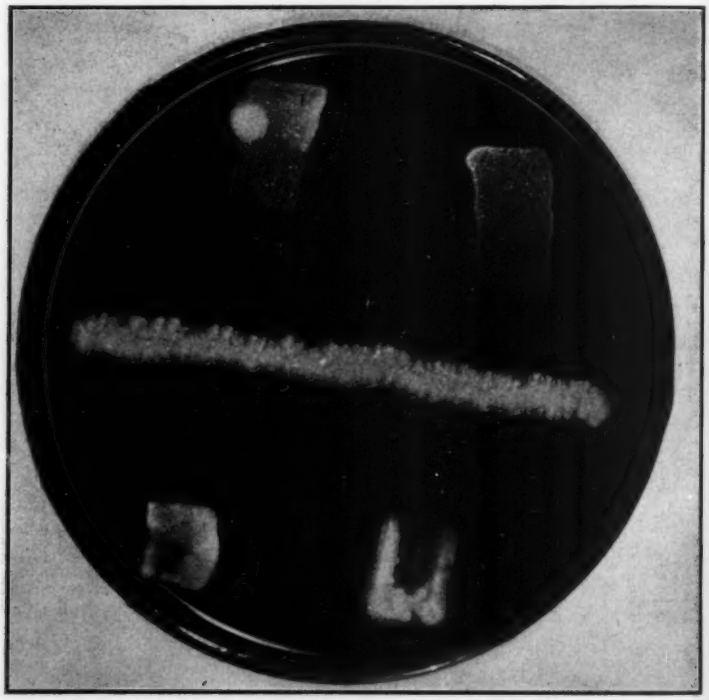

FIG. 2. Streak test showing antagonism of four pathogenic fungi by a soil actinomyces.

43 per cent, respectively, exhibited antifungal properties. The fact that approximately four times as many filtrates were active by the diffusion method as by the dilution technic would appear to indicate that a rather high percentage of the filtrates contained heat-labile agents which were destroyed in the heating process. That this was not true was demonstrated by simultaneously cup-assaying

TABLE I

THE DISTRIBUTION OF ANTIFUNGAL PROPERTIES AMONG SOIL ACTINOMYCETES ISOLATED AT RANDOM

Source of Cultures	Agar-Streak Test					Agar-Dilution Test						Agar-Diffusion Test				
	Total Active Cultures*	Antagonists against†				Total Active Cultures	Filtrates					Total Active Cultures	Filtrates			
							Total Number Active	Inhibition of					Total Number Active	Inhibition of		
		CA	CN	TG	TP			CA	CN	TG	TP			CA only	CN only	CA and CN
Forest soil	19/38	15	18	18	17	4/19	4/47	0	4	1	2	13/19	22/47	3	11	8
Leaf compost	12/25	10	6	6	11	0/10	0/16	0	0	0	0	4/10	4/16	1	3	0
Cornfield soil	18/36	14	15	10	8	1/11	2/23	0	2	0	0	4/10	6/20	3	2	1
Peat	10/18	9	8	8	8	2/8	3/20	0	3	1	1	5/8	11/21	3	4	4
Field soil	28/53	20	18	20	22	3/17	7/35	4	7	6	6	10/18	19/36	4	7	8
Miscellaneous garden soils	13/28	10	9	10	11	0/4	0/12	0	0	0	0	4/7	4/15	1	3	0
Antiphage actinomycetes	24/45	17	4	7	13							0/1	0/0	0	0	0
Total	124/243	95	78	79	90	10/69	16/153	4	16	8	9	40/73	66/155	15	30	21
Per cent	51					15	10					55	43			

* Numerator = number of cultures or filtrates active on one or more media against at least one fungus; denominator = total number of cultures or filtrates tested.

† CA = *Candida albicans;* CN = *Cryptococcus neoformans;* TG = *Trichophyton gypseum;* TP = *Trichophyton rubrum* (*purpureum*).

heated and unheated samples of twenty filtrates previously shown to be active by the diffusion but not by the dilution method. The results of these tests, in which the preparations proved to be heat-stabile, indicated that the cup assay was more sensitive than the dilution method.

The influence of composition of the media on the antagonism of fungi by soil actinomycetes is shown in Table II. In general, it

TABLE II

THE INFLUENCE OF COMPOSITION OF MEDIA ON THE DISTRIBUTION OF ACTINOMYCETES ANTAGONISTIC TO PATHOGENIC FUNGI *

Source of Cultures	Agar-Streak Test															
	Nutrient Agar Antagonists against†			Glucose-tryptone Agar Antagonists against				Glycerol-yeast Extract Agar Antagonists against				Percentage of Total Streaks Active against‡			
	CA	TG	TP	CA	CN	TG	TP	CA	CN	TG	TP	CA	CN	TG	TP
Forest soil	14	16	15	14	16	13	14	10	14	13	12	33	40	36	36
Leaf compost	8	3	7	1	2	3	4	4	6	4	5	20	16	15	25
Cornfield soil	8	6	7	7	9	6	5	8	14	4	5	21	32	15	16
Peat	6	5	4	3	5	6	6	6	6	7	7	28	31	33	32
Field soil	13	11	11	12	15	18	17	15	16	17	18	25	29	29	28
Miscellaneous garden soils	7	8	8	6	10	11	12	7	7	7	9	24	20	30	34
Antiphage actinomycetes	15	0	3	1	1	1	7	2	3	7	10	13	4	6	15
Total	71	49	55	44	58	58	65	52	66	59	66				
Per cent	29	20	22	18	24	24	27	21	27	24	27				

* See Table I for total number of cultures tested from each source.

† CA = *Candida albicans;* CN = *Cryptococcus neoformans;* TG = *Trichophyton gypseum;* TP = *Trichophyton rubrum* (*purpureum*).

‡ $\dfrac{\text{(Total antagonists (Table II)) (100)}}{\left(\begin{matrix}\text{Total cultures}\\ \text{tested (Table I)}\end{matrix}\right) \times \left(\begin{matrix}\text{Number of test}\\ \text{media}\end{matrix}\right)}$ = Per cent of total streaks active.

appears that each fungus was antagonized by more or less the same percentage of actinomycetes on all three media. The results in Table II indicate that for these four fungi, at least, there is no great variation in sensitivity which approaches the difference between Gram-positive vis-à-vis Gram-negative bacteria (**11, 15**). Actually, there were some streak tests in which the actinomycetes exhibited activity against two or three of the four test fungi; only an occasional microorganism was active against but one of the

pathogens. On the other hand, the tests with culture filtrates (Table I) showed that *Cryptococcus neoformans* was in general the most sensitive of the four fungi; *Candida albicans* was the most resistant; the two species of *Trichophyton* were intermediate and both of the same order of sensitivity. This variation in susceptibility to culture filtrates is similar to that obtained when such preparations are tested against bacteria.

Table III presents the results of a more detailed study of broth filtrates of four of the antagonistic actinomycetes. The dependence of the production of antifungal agents on composition of the medium, the rate of production of the active substances, and the selective nature of the inhibitive effect are indicated here. The nutrient broth was apparently a much poorer substrate than the glucose-tryptone or glycerol-yeast extract media. It is of interest to mention here that none of these culture filtrates exhibited any activity against *Bacillus subtilis, Escherichia coli,* or *Mycobacterium phlei* by the agar-dilution method.

From the antifungal spectra of the culture filtrates (Table III), it appears that cultures *No. 47204* and *No. 47205* may produce the same or similar agents, but that microorganism *No. 4779* is decidedly different, especially with respect to activity against *Candida albicans* and lack of inhibition of the trichophyta by the agar-dilution test.

Table II shows that the forest soil contained a somewhat higher percentage of antagonistic actinomycetes than did the field soil, peat, leaf compost, cornfield soil, or miscellaneous garden soils. The actinomycetes that had been found to be antagonistic to bacteriophages were the least active group against the pathogenic fungi. Approximately the same order of activity was obtained when these cultures were tested against nonparasitic fungi. From data not reported here, only eight of fifty-four antiphage actinomycetes were antagonistic on one or more media to a *Trichoderma* sp. and a strain of *Aspergillus* which appeared to be *Aspergillus unguis*. In contrast to this, thirty-five of the actinomycetes antagonized *Streptomyces griseus*. The lower activity of the antiphage microorganisms may have been due to the fact that these cultures had been maintained on dextrose-asparagin agar for approximately one year, whereas the other microorganisms were fresh

TABLE III

ANTIFUNGAL ACTIVITY OF FOUR ANTAGONISTIC SOIL ACTINOMYCETES *

Culture No.	Broth Medium	Incubation Period, Days	Agar-dilution Test, Units per ml. against†				Agar-diffusion Test, mm. Zone of Inhibition against	
			CA	CN	TG	TP	CA	CN
47379	Nutrient broth	9	0	0	0	0	0	18
		13	0	0	0	0	0	20
		16	0	0	0	0	0	18
	Glucose-tryptone	9	0	75	0	0	0	>35
		13	0	50	0	0	10	>35
		16	0	5	0	0	0	20
	Glycerol-yeast extract	9	0	10	0	0	10	>35
		13	0	75	0	0	10	>35
		16	0	60	0	0	12	>35
47204	Nutrient broth	9	0	0	0	0	20	>35
		13	0	5	0	0	20	35
		16	0	0	0	0	16	32
	Glucose-tryptone	9	0	20	5	5	18	>35
		13	0	25	5	5	20	35
		16	0	25	5	10	18	32
	Glycerol-yeast extract	9	0	25	5	5	16	32
		13	0	15	0	0	16	32
		16	0	5	0	0	16	30
47205	Nutrient broth	9	0	5	0	5	12	30
		13	0	5	0	0	12	30
		16	0	5	0	0	18	32
	Glucose-tryptone	9	0	20	10	5	14	35
		13	0	75	5	10	18	35
		16	0	60	15	15	18	35
	Glycerol-yeast extract	9	0	15	0	0	18	30
		13	0	30	5	5	18	35
		16	0	25	0	5	18	35
4779	Glucose-tryptone	9	0	5	0	0	10	15
		13	0	10	0	0	12	18
		16	0	10	0	0	10	18
	Glycerol-yeast extract	9	10	30	0	0	16	22
		13	20	75	0	0	16	24
		16	10	75	0	0	16	24

* CA = *Candida albicans;* CN = *Cryptococcus neoformans;* TG = *Trichophyton gypseum;* TP = *Trichophyton rubrum* (*purpureum*).

† Number of units = reciprocal of the final dilution of culture filtrate per ml. of agar that just inhibits growth of the test microorganism.

isolates. It is well known that actinomycetes when transferred on synthetic media for long periods of time tend to lose certain properties.

In order to obtain information on the relative abundance of bacteria and fungi in soil antagonistic to pathogenic fungi, two

selective technics were employed. The first method required that the test culture grow appreciably more slowly than the majority of soil microorganisms. *Trichophyton rubrum* (*purpureum*) proved to be the only one of the four fungi satisfactory in this respect. Table IV presents the data obtained when the forest soil, leaf

TABLE IV

DISTRIBUTION OF SOIL MICROORGANISMS* ANTAGONISTIC TO *Trichophyton rubrum* (*purpureum*)

Source of Culture	Microorganisms	Nutrient Agar	Glycerol-yeast Extract Agar	Dextrose-asparagin Agar
Forest soil	Actinomycetes	4.3 †/30	2.5/22.5	2.5/5.5
	Bacteria	0.3 †/20	0.1/64	0/10
	Fungi	0/0	0/2	0/4
Leaf compost	Actinomycetes	2.5/56	0.6/48	1.8 †/32
	Bacteria	0.2 ‡/TN	0.6/TN	0.3 †/162
	Fungi	0/0.5	0/2	0.5 †/1
Field soil	Actinomycetes	—	1.8/36	7.8/24.5
	Bacteria	—	0/31	0/33
	Fungi	—	0/1.5	0/3

* Counts × 10^5 = numbers per gm. of moist soil. Numerator = antagonistic colonies. Denominator = total colonies.
† Values representing single plate counts.
‡ TN = colonies too numerous to count.

compost, and field soil were plated in the three agar media seeded with *Trichophyton rubrum* (*purpureum*). The total number of microorganisms in each soil was determined by simultaneously plating the same soil dilutions with fungus-free agar. Unless otherwise indicated, the values represent averages from three to six plates.

In another type of experiment, the soils and leaf compost were

plated and counts made after from five to six days' incubation, following which the plates were carefully flooded with fungus-seeded glucose-tryptone agar. In figure 3, which illustrates a modification of this technic, the secondary layer of agar had been seeded with both *Candida albicans* and *Cryptococcus neoformans*,

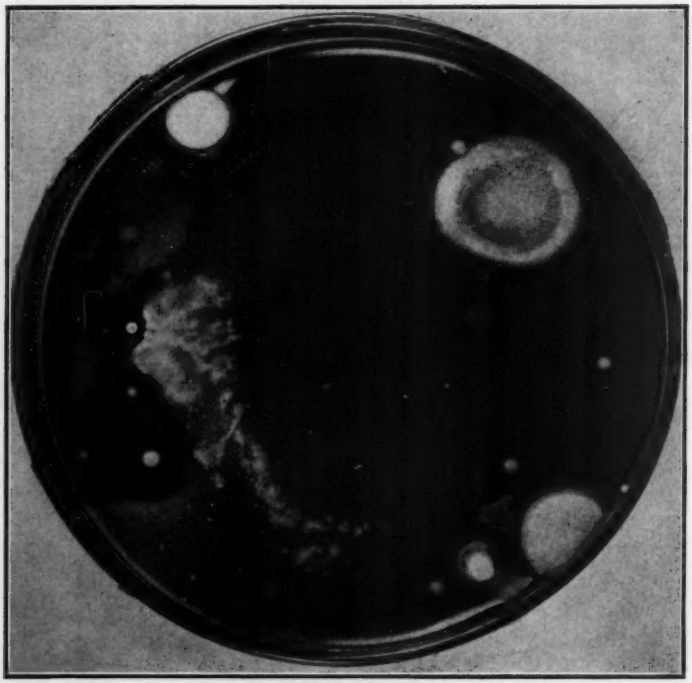

FIG. 3. Simultaneous antagonism of both *Candida albicans* and *Cryptococcus neoformans* by soil actinomycetes.

so that the zones surrounding six colonies of actinomycetes indicate simultaneous antagonism of both pathogenic fungi. The results obtained by this method with *Candida albicans*, *Cryptococcus neoformans*, and *Trichophyton gypseum* are presented in Table V. All values represent average counts from three to six plates selected because of well-isolated colonies and the absence of spreading

spore-formers at the time total counts were made and the second layer of seeded agar was poured.

It is evident from the data in Tables IV and V that the actinomycetes in general contain a much higher percentage of forms antagonistic to the pathogenic fungi than do the bacteria. Since fungi are considerably less numerous than actinomycetes and bac-

TABLE V

DISTRIBUTION OF SOIL MICROORGANISMS ANTAGONISTIC TO *Candida albicans*, *Cryptococcus neoformans*, AND *Trichophyton gypseum* *

Source of Culture	Microorganisms	Nutrient Agar			Glycerol-yeast Extract Agar			Dextrose-asparagin Agar		
		CA	CN	TG	CA	CN	TG	CA	CN	TG
Forest soil	Actinomycetes	8.5/21.8	9.5/18.8	4.4/19	8/21	15.3/27.3	10/24.3	0.8/19.2	2/23.3	0/21
	Bacteria	0/14.5	0/13.3	0/23.3	0.3/65.8	0/41	0/13.5	0.2/24	0/18.5	0/11.5
	Fungi	0.3/1.5	0/0.5	0/0.8	1.0/3.8	0/1.5	0/2.5	0/4.0	0/4.3	0.5/5.8
Leaf compost	Actinomycetes	25/40	27/73	23/37	25/30	17/47	20/32	3.5/8	3/16.5	—
	Bacteria	0/345	7/660	6/1490	2/1240	7/1080	0/1360	0/385	0/416	—
	Fungi	0/0	0/3	0/3	2/14	0/16	2.5/32.5	0/1.4	0/2.3	—
Field soil	Actinomycetes	6.5/38	4.8/80.5	3.5/42.8	4.8/42.5	8.5/47.5	5/46.4	2.3/22.5	3.0/21.0	2.8/37.8
	Bacteria	0.5/41	0.5/20.3	0.5/38.8	0.3/75	0/43	0/83.6	0/24.5	0/18.3	0/49.6
	Fungi	0/1.5	0/0	0/1.3	0.5/1.5	0/2	0.6/1.6	0.5/2.3	0.3/2.3	0.2/1.8

* CA = *Candida albicans;* CN = *Cryptococcus neoformans;* TG = *Trichophyton gypseum;* TP = *Trichophyton rubrum* (*purpureum*).

teria, relatively few fungus colonies were present on plates at those dilutions most suitable for counting the bacteria and actinomycetes. Nevertheless, the limited data do indicate that the percentage of antagonistic forms among the fungi was much closer to that for actinomycetes than for bacteria.

The number of antagonistic actinomycetes for many of the counts

in Tables IV and V is actually greater than indicated. The reason is that often a single large zone or several confluent zones surrounded a number of actinomyces colonies that had grown close to one another. Moreover, the actinomycetes on some plates were overgrown by soil fungi so that inhibition of the test pathogen could not be clearly discerned even if there had been antagonism. In general, nearly all of the subsurface bacterial colonies, particularly on the dextrose-asparagin agar, were very small. Nevertheless, it is unlikely for two reasons that the low percentage of active bacteria was due to limited growth. First, many actinomyces colonies equally small in size were surrounded by fairly large zones of inhibition. Second, there were few antagonists even among the large, well-developed bacterial colonies on the surface of the agar.

From data not presented here, nearly all of twenty-two actinomycetes isolated from colonies that antagonized *Cryptococcus neoformans* in the secondary agar layer poured over soil plates, were shown to be active when streaked on the three media against the four test fungi; of fifteen actinomycetes picked from colonies showing similar inhibition against *Candida albicans,* eleven showed good activity against the pathogens when streak-tested on the three media. The others were of a low order of activity. These relatively high percentages of active microorganisms as contrasted with the corresponding values in Table I illustrate the advantage of selective procedures over random isolation in the search for antagonists. When tested by the agar streak, six of ten fungi isolated from colonies inhibitive to *Candida albicans* or *Cryptococcus neoformans* gave varying degrees of activity against the test microorganisms on the three different media. By the agar-dilution technic, only one of six cultures tested yielded filtrates that were active when heat-treated. The unheated filtrates of all six microorganisms, however, were active against *Candida albicans* or *Cryptococcus neoformans,* or both, by the cup assay. When streaked against one another, the four pathogenic fungi exhibited no inhibitive properties. It might also be mentioned that three out of six cultures of *Bacillus subtilis* selected at random and streak-tested were inactive, while three inhibited one or more of the fungi on at least one medium; three Gram-negative bacilli were completely inactive by the streak test.

SUMMARY AND CONCLUSIONS

The results of a study of various soils and compost material for the distribution of microorganisms antagonistic to fungi pathogenic for man are presented. Methods of random isolation and selective plating were employed to isolate the antagonists. The agar-streak method in which three media were employed, the agar-dilution and agar-diffusion tests with a glucose-tryptone agar were used to demonstrate antifungal activity. *Candida albicans, Cryptococcus neoformans, Trichophyton gypseum,* and *Trichophyton rubrum* (*purpureum*) were used as the test fungi.

One hundred and twenty-four or 51 per cent of two hundred and forty-three actinomycetes were found to be antagonistic by the agar-streak method to one or more of the four test fungi. One hundred and ninety-eight of the cultures tested were isolated from various soils and forty-five were previously reported to be antagonistic to bacteriophage.

The data obtained from the selective plating procedures for isolating antifungal microorganisms show that the percentage of antagonistic bacteria was considerably less than that of the antagonistic actinomycetes. The data for soil fungi, although limited, indicate a distribution of antagonistic forms in between the values for bacteria and actinomycetes and probably closer to that of the latter.

The assays of culture filtrates of those actinomycetes active by the streak test revealed that 15 per cent of the microorganisms were active by the agar-dilution test, whereas 55 per cent exhibited inhibition by the diffusion method. The greater activity by the diffusion assay indicates this to be the more sensitive of the two methods.

The nutrient broth was decidedly inferior to the glucose-tryptone and glycerol-yeast extract media with respect to production of culture filtrates with antifungal activity, although the percentage of actinomycetes antagonistic to each of the test fungi was comparable on the three agar media used. *Cryptococcus neoformans* was found to be the more sensitive, and *Candida albicans* the more resistant to the active culture filtrates by the agar-dilution procedure.

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PRELIMINARY OBSERVATIONS ON THE MORPHOLOGY AND CYTOLOGY OF AN UNDESCRIBED HETEROBASIDIOMYCETE FROM WASHINGTON STATE [1]

GEORGE NYLAND [2]

(WITH 1 FIGURE)

A curious fungus was isolated from red raspberry leaves severely attacked by western yellow rust (*Phragmidium rubi-idaei* (DC.) Karst.).[3] In culture the fungus buds in a yeast-like manner and also produces spores by repetition. These spores, here called sporidia, are produced on sterigmata, which may develop either from similar sporidia or from the yeast-like cells. The sporidia are forcibly abjected in the same manner as the basidiospores of many Basidiomycetes.

Fifteen monosporidial cultures were made from the original isolate and all developed in the same manner. The original sporidium produced a bud within a few hours. When the budded cell attained approximately the size of the original sporidium, it separated and in turn produced a bud. Each cell so formed budded vigorously after the manner of yeasts until a colony barely visible to the unaided eye was formed. Then it was observed that, in addition to continued budding, the surface cells of the colony produced aerial sterigmata. On these, asymmetrical sporidia were formed and forcibly discharged (FIG. 1, *A–D*). Some of these discharged sporidia produced other cells by budding and others produced secondary sporidia on sterigmata. Apparently each sporidium is capable of doing either.

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[2] Assistant Professor and Assistant Plant Pathologist, State College of Washington.

[3] The fungus was isolated by Dr. Folke Johnson.

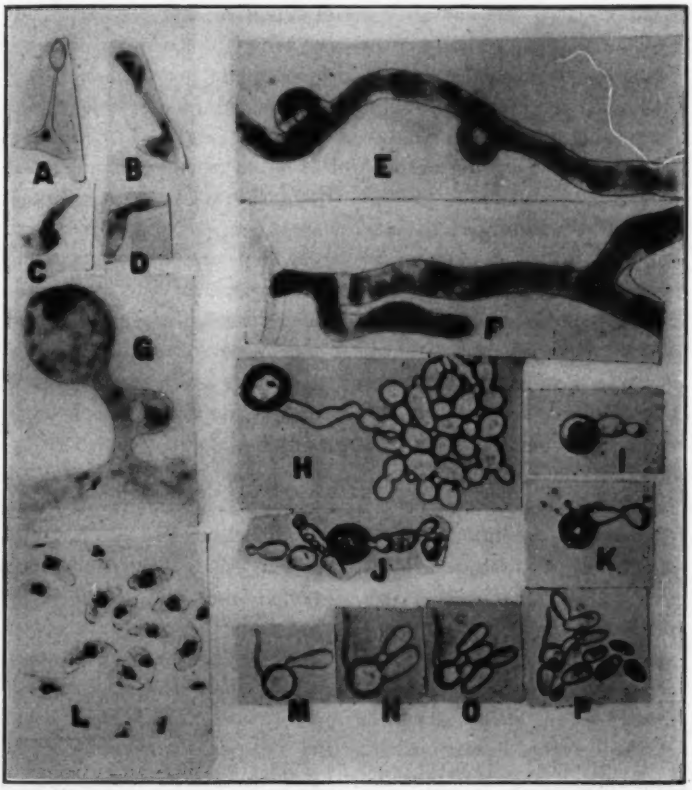

FIG. 1. *A–D*, germinating spores producing secondary spores by repetition. *D* shows 2 nuclei in the sterigma, × 1,000. *E–F*, mycelium showing clamp connections and binucleate cells, × 1,400. *G*, young resting spore receiving a second nucleus through the clamp connection on the stalk, × 1,700. *H–K*, germinating resting spores producing yeast-like cells which later produced spores on sterigmata by repetition and also mycelium, × 600. *L*, spores that were forcibly abjected from sterigmata onto a slide and stained with iron-alum haematoxylin, × 1,000. *M–P*, stages in the germination of a resting spore. Photomicrographs taken at 2 hour intervals, × 600.

When colonies started from a single sporidium were approximately three days old it was observed that mycelium was growing out from the margins of the colonies. The mycelium was septate, with a clamp connection at almost every septum (FIG. 1, *E–F*).

Approximately one day after the mycelium was first observed, it was noticed that resting spores were being formed some distance behind the terminal cells. These spores were produced on short stalks and were typically terminal but sometimes intercalary. A clamp connection was produced on each stalk (FIG. 1, *G*). Usually a hyaline, hyphal projection was observed to extend from the apex of each spore that was produced terminally. The resting spores are hyaline and thin-walled when young, but become golden brown and thick-walled when mature.

Early attempts to germinate the resting spores were unsuccessful. Germination was finally induced by incubating agar cultures at 35° C. for six days or longer. The spores germinated by producing a short germ tube or primary cell from which hyaline, yeast-like cells were budded (FIG. 1, *H–K*; *M–P*). These cells in turn budded, and development proceeded in the same manner as already described, including the formation of mycelium and resting spores.

The cells that are produced by budding, as well as the sporidia produced by repetition, are typically uninucleate (FIG. 1, *L*). In stained preparations occasional binucleate cells and sporidia were observed. The mycelium that develops from these colonies of predominantly uninucleate cells and sporidia is typically composed of binucleate cells (FIG. 1, *E–F*). The resting spores are uninucleate when young but become binucleate soon after the clamp is formed on the stalk bearing the spore (FIG. 1, *G*). The two nuclei in the spore fuse immediately and the spore assumes the heavy wall and golden brown color shortly thereafter. The cells formed as a result of the germination of the resting spores, and the sporidia formed subsequently, are uninucleate. It is believed that these yeast-like cells and the sporidia contain a diploid nucleus. The mycelium is believed to be dikaryotic, and fusion of the two nuclei (haploid?) occurs in the immature resting spore. Just where reduction division takes place, or if it occurs at all, is not known at the present time.

The stage in the life cycle of this fungus represented by the yeast-like cells and the sporidia agrees with the described characters of the genus *Sporobolomyces*.[4] However, because of the presence of mycelium and resting spores, this fungus cannot be placed in this genus. Cultures of all the described species of *Sporobolomyces*

were obtained from the Centraalbureau at Delft, Holland, and subcultures have been examined repeatedly by the writer. The production of true mycelium has not been observed in any of these cultures.

Since the fungus, as far as is known, lacks the faculty of parasitism, the author hesitates to place it in the Ustilaginales. It apparently lacks an organized fruit body, at least in culture, which would seem to exclude it from the Tremellales. It has some characters in common with both orders, but, at the present and pending further study, it is simply considered to be a Heterobasidiomycete, the affinities of which are unknown. It is anticipated that in a later paper a detailed description of the fungus and a proposed name will be presented. Further study is required to provide answers to some of the puzzling features of the nuclear history.

[4] Kluyver, A. J., and C. B. van Niel. 1924–1925. Über spiegelbilder erzeugende Hefenarten und die neue Hefengattung Sporobolomyces. Centralblatt für Bakteriologie, Parasitenkunde, und Infectionskrankheiten. Abt. 2, **63**: 1–20.

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THE GENUS PLECTANIA AND ITS SEGREGATES IN NORTH AMERICA

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(WITH 12 FIGURES)

The genus *Plectania* was established by Fuckel (**1869–70**) and antedates the genus *Sarcoscypha* Saccardo by twenty years. Fuckel included two species, *Peziza coccinea* Fr. and *P. melastoma* Fr. The latter is now in the genus *Rhizopodella* Boudier. Seaver (**1928**) in his treatment of *Plectania* for North America included three species in addition to *P. coccinea*. Two of them, *Peziza floccosa* and *P. occidentalis*, were described by Schweinitz (**1834**) from specimens found in the United States, and since that date they have been collected frequently in this country. The fourth species, *Peziza protracta* Fr., is rarely collected, although it has a wide distribution to judge from published reports. It appears to be confined to the northern hemisphere, and further limited to cold areas. Only about fifty reports have been located in the literature. Six of these are from North America.

At least sixteen additional species, which have been reported from North America in the genus *Sarcoscypha*, have either already been transferred to other genera or are so inadequately described that their identity is uncertain. This investigation deals with the four species just named. It was initiated by a collection from Isle Royale, Michigan, that was made November 15, 1941, by Mr. and Mrs. H. E. Bailey. It was tentatively named *Plectania protracta* (Fr.) Gelin although it was recognized that several of its characters were distinctly different from those encountered in other species of *Plectania*. Further study, including the examination of six additional collections from both North America and Europe, together with a résumé of the literature, convinced me that this taxonomic position was untenable.

* Papers from the Herbarium of the University of Michigan.

In the course of the study, information was obtained on other species which led to the revision set forth in this paper. One phase of the study centers around the paraphyses. It was found that in the four species under consideration, they have been erroneously described or that correct descriptions have been disregarded. Paraphyses offer important diagnostic generic characters as is evident in taxonomic literature dealing with Discomycetes. In *Orbilia, Otidea, Apostemidium* and *Ionomidotis* they are universally regarded as having special significance, and in many other genera they offer much help to the taxonomist. Consequently, accurate descriptions of them are necessary.

I am greatly indebted to some of my colleagues for the opportunity of studying collections pertinent to this investigation. Thanks are due to Dr. F. J. Seaver for the loan of collections of *Plectania protracta* and *P. floccosa* from the Herbarium of the New York Botanical Garden; to Dr. J. A. Stevenson and Miss Edith Cash for slides of *Peziza floccosa* from the ex-Michener Herbarium, Bureau of Plant Industry, United States Department of Agriculture, and also for additional collections of *P. floccosa;* to Miss Ruth Patrick for fragments of *Peziza floccosa* from the Schweinitz Herbarium, Philadelphia Academy of Science. Both the slides and the fragments are presumably of the type of *P. floccosa.* The place of collection is Nazareth, Pennsylvania. Dr. Homer D. House provided the type of *Peziza Dudleyi* Peck from the Herbarium of the New York State Museum, and Dr. Rolf Singer supplied the type collection of *Sarcoscypha javensis* Syd. Miss Louise Drosall contributed specimens of *Sarcoscypha floccosa* and *S. protracta* from the Daisy Hone Herbarium in the Herbarium of the Division of Plant Industry, University of Minnesota.

KEY TO PLECTANIA AND ITS SEGREGATES

1. Paraphyses forming a reticulum.....*Anthopeziza.*
1. Paraphyses not forming a reticulum.....2.
2. Gelatinous layer present in stipe and apothecium.....*Microstoma.*
2. Gelatinous layer lacking.....*Plectania.*

PLECTANIA Fuckel

Apothecia stipitate or sometimes nearly sessile, at first subglobose, expanding to shallow cup-shaped or discoid but with margin

usually enrolled, externally somewhat hairy; hymenium bright colored inclined to scarlet; asci 8-spored; spores ellipsoid, hyaline to slightly yellowish, smooth; paraphyses forked or branched sparingly.

Type species *Peziza coccinea* Fr.

PLECTANIA COCCINEA (Fr.) Fuck. Symb. Myc. 324. 1869 (FIGS. 8–9).

Peziza coccinea Fries. Syst. Myc. **2**: 79. 1822.
Lachnea coccinea Gill. Champ. Fr. Discom. 66. 1880.
Sarcoscypha coccinea Sacc. Syll. Fung. **8**: 154. 1889.
Peziza Dudleyi Peck. Ann. Rept. N. Y. State Mus. **47**: 23. 1894.
Geopyxis coccinea Massee. Brit. Fung. **4**: 377. 1895.

Apothecia gregarious, subsessile to short stipitate, cup-shaped, margin enrolled strongly when dry, 2–4 cm. in diameter, hymenium scarlet, outside creamy white, floccose with more or less matted hyaline hairs, outside often veined and ridged when dry; stipe stout, of variable length depending upon the depth at which the sticks to which it is attached are buried; asci long cylindrical, tapering into a short stem-like base, 300–375 × 14–16 μ; spores hyaline, narrowly ellipsoid, usually containing two large oil drops, 24–32(40) × 12–14 μ, uniseriate; paraphyses flexuous, branched usually by forking near the base, uniformly slender, containing red coloring matter when fresh, fading on drying. No blue coloration with iodine.

On buried or partially buried sticks; early spring; North America and Europe.

MATERIAL EXAMINED: UNITED STATES. **California**: Inverness, March 1931, W. B. Cooke, (*Mich.*); Marin Co., March 20, 1939, T. T. McCabe, (*Mich.*). **Iowa**: Decorah, April 1880, E. W. Holway, *N. A. F. 434*, (*Mich.*). **Michigan**: Ann Arbor, April 1893, E. R. Wolfenden, (*Mich.*); Ann Arbor, May 14, 1893, Lola Conrad, (*Mich.*); Mason, April 1, 1929, B. Kanouse, (*Mich.*); Ann Arbor, June 1932, A. H. Smith, (*Mich.*); Lakeland, June 20, 1935, A. H. Smith, (*Mich.*); Milford, April 20, 1940, A. H. Smith, (*Mich.*); Cass Co., April 4–14, 1946, Elizabeth Halfert, (*Mich.*); Chelsea, May 19, 1947, Morten Lange, (*Mich.*). **New York**: Buttermilk Gorge, Ithaca, April 14, 1903, C. H. Kauffman, (*Mich.*). **Ohio**: Oberlin, May 1924, F. O. Grover, (*Mich.*). **Tennessee**: Tremont, March 18, 1934, L. R. Hesler, *No. 3886*, (*Mich.*); "Chimneys," Great Smoky Mts. Nat. Park, March 31, 1929, L. R. Hesler, *No. 2098*, (*Mich.*); Gatlinburg, March 27, 1940, Mrs. Jensen, (*Mich.*). **West Virginia**: Lafayette Co., March 20, 1893, L. W. Nuttall, *No. 852*, (*Mich.*).
CANADA: Old Chelsea, Quebec, April 25, 1935, I. L. Conners, (*Mich.*)

EUROPE: Bosnien, April 1901, v. Höhnel, (Rehm: Ascomyceten, *No. 1404*), (*Mich.*).

PLECTANIA OCCIDENTALIS (Schw.) Seaver. North American Cup-fungi p. 193. 1928 (FIGS. 5–7).

Peziza occidentalis Schw. Trans. Am. Phil. Soc. II. **4**: 171. 1834.
Peziza hesperidea Cooke and Peck. Grev. **1**: 5. 1872.
Geopyxis occidentalis Morgan. Jour. Myc. **8**: 188. 1902.
Sarcoscypha occidentalis Sacc. Syll. Fung. **8**: 154. 1889.
Geopyxis hesperidea Sacc. Syll. Fung. **8**: 63. 1889.

Apothecia stipitate, gregarious or cespitose, 1 cm. in diameter, shallow cup-shaped, margin enrolled when dry, hymenium scarlet, drying "capucine yellow" (R.) (**1912**) to "orange buff," outside smooth; stipe 1–3 cm. long, sometimes surrounded at the base with hyaline hyphae; asci cylindrical, 250–300 × 16–18 μ, 8-spored, spores in one row in the asci; spores hyaline to slightly yellowish, smooth, ellipsoid, containing two large oil drops, 18–20 × 10–12 μ; paraphyses flexuous, branched, forked near the base, sometimes branched more than once. No blue coloration in iodine.

On buried or partially buried sticks; type locality Muskingum, Ohio; United States.

MATERIAL EXAMINED: **Kentucky**: Harlan, Sept. 8, 1916, F. B. Cotner, (*Mich.*). **Maine**: Kittery Point, Aug. 1920, R. Thaxter, (*Mich.*), Farlow Herb., *No. 641*. **Michigan**: Ann Arbor, June 3, 1893, L. N. Johnson, (*Mich.*); Ann Arbor, May 26, 1894, L. N. Johnson, (*Mich.*) and Ann Arbor, Sept. 15, 1894, (*Mich.*); Ann Arbor, June 24, 1905, C. H. Kauffman, (*Mich.*); Ann Arbor, June 10, 1906, C. H. Kauffman, (*Mich.*); Ann Arbor, July 5, 1915, C. H. Kauffman, (*Mich.*); Whitmore Lake, July 25, 1915, E. B. Mains, (*Mich.*); Whitmore Lake, July 27, 1915, C. H. Kauffman, (*Mich.*); Ann Arbor, July 29, 1927, C. H. Kauffman, (*Mich.*); Whitmore Lake, July 10, 1929, A. H. Smith, (*Mich.*); Ann Arbor, July 11, 1929, B. B. Kanouse, (*Mich.*); Pinckney (George Reserve), July 2, 1931, E. B. Mains, (*Mich.*); Silver Lake, June 17, 1935, A. H. Smith, *1343*, (*Mich*); Sharon Hollow, June 1937, A. H. Smith, (*Mich.*); Ann Arbor, June 13, 1942, A. H. Smith, *18336*, (*Mich.*). **Pennsylvania**: West Chester, N.A.F. *436*. Aug. 1879, Haines, Everhart & Wood, (*Mich.*); Lebanon Co., June 15, 1904, C. H. Kauffman, (*Mich.*). **Tennessee**: Knoxville, June 25, 1929, W. A. Anderson, (*Mich.*). **West Virginia**: Fayette Co., Aug. 8, 1893, L. W. Nuttall, *591*, (*Mich.*).

In the genus *Plectania* the situation regarding paraphyses as presented in the literature is confusing. A few of the contradictory citations will be briefly mentioned. To start with Fuckel's description, we find that not only the genus but also the type spe-

cies, *P. coccinea,* is credited with having filiform paraphyses. Seaver (**1928**) accepted this concept for the genus. Of the two species *P. coccinea* and *P. occidentalis,* respectively, he states, "paraphyses slightly enlarged above" and "paraphyses slender, slightly thickened above." His illustrations do not show branching. In an earlier paper (**1904**) he described and illustrated *S. coccinea* as having linear paraphyses. Of *S. occidentalis* he stated that they were linear and illustrated them as forked. In his Iowa Discomycetes (**1910**) the illustrations were unchanged and there were no comments concerning the paraphyses. Rehm (**1895**) reported *Sarcoscypha coccinea* as having paraphyses "gabelig, getheilt" and so illustrated them. Boudier (**1905–1910**) likewise shows them to be forked in *S. coccinea.* The American species *P. occidentalis* likewise has forked paraphyses. For the sake of accuracy this data concerning branching must be incorporated in the generic and specific descriptions, or the confusion will be perpetuated.

There remain species, still known in the genus *Sarcoscypha,* chiefly European, that should be restudied. It is possible that some described as having filiform paraphyses will be found to have them branched. A species from Java, *Sarcoscypha javensis* Syd. the type of which the writer examined, has branched paraphyses as Sydow described them.

MICROSTOMA Bernstein

Apothecia stipitate, arising from a hard, black pseudorhiza buried in the soil, usually attached to roots or wood; aerial stipe long, usually branched, covered with hairs, stipe and cups containing a layer of gelatinous hyphae; cups at first subglobose to pyriform, opening by a small pore, becoming expanded, finally laciniate; asci cylindrical, 8-spored; spores large, ellipsoid to fusoid, hyaline to slightly yellowish; paraphyses dichotomously branched several times.

Type species *Peziza protracta* Fr.

Microstoma protracta (Fr.) comb. nov.

Peziza protracta Fr. Nov. Symb. Myc. Mantissa. Act. R. Soc. Sci Uppsala 3. Ser. 1: 230. 1851. (FIGS. 3–4.)
Peziza cruciata Fr. Nov. Symb. Mantissa 229. 1851.

Microstoma hiemale Bernst. Nova Acta Acad. Caes. Leop.-Carol. Natur. 23. **2**: 649. tab. 61. 1852; Milde, J. von. Bot. Zeit. **10**: 208. 1852.
Peziza mirabilis Borsz. Fungi Ingrici p. 61. t. 4, 5. 1857.
Sclerotinia baccata Fuck. Symb. Myc. 331. tab. 4. fig. 38. 1869.
Peziza hiemalis Karst. Myc. Fennica pars prima p. 44. 1871.
Sclerotinia hiemalis Fuck. Symb. Myc. Nachtr. II. p. 65. 1873.
Scypharia coccinea var. *hiemalis* Quél. Enchir. fung. 282. 1886.
Lachnea mirabilis Phill. Grev. **18**: 83. 1889.
Sarcoscypha cruciata Sacc. Syll. Fung. **8**: 154. 1889.
Sarcoscypha protracta Sacc. Syll. Fung. **8**: 155. 1889.
Sarcoscypha alpina E. & E. Bull. Torrey Club **24**: 281. 1897.
Plectania hiemalis (Nees and Bernst.) Seaver. North Am. Cup-fungi p. 193. Pl. 19. 1928.
Plectania protracta Gelin. Det. Kgl. Norske Viden. Selsk. Forh. Bd. 10. Nr. **52**: 194. 1938.
Plectania protracta Imai. Bot. Mag. 52. No. **61**: 362. 1938.

Apothecia stipitate, arising from an immersed, hard, elongate, pseudorhiza attached to buried wood or roots of trees, perennial, aerial stipes branched usually several times, giving rise to as many as eleven apothecia, aerial stipes long, slender, 2–6 cm. long, depending upon the depth to which the pseudorhiza is buried, lower one-half dark colored, upper portion light colored, covered with hyaline hairs, containing a layer of gelatinous tissue; cups 1–2 cm. in width, 1–2 cm. in depth, at first subglobose to pyriform, expanding, opening to deep vase-shaped, often with a flat collar, finally laciniate, opening by a small pore surrounded by a row of stiff, short, hyaline hairs, lower part of the cup covered with hyaline hairs, upper part smooth, externally bright orange-red, interior vivid rose-red, fading when dry, hypothecium consisting of a subhymenial layer of densely matted hyphae, a middle layer of gelatinous hyphae and an excipular layer the outermost tissue of which gives rise to the excipular hairs; asci cylindrical, 200–275 × 20–23 μ, the base sharply contracted into long, slender hyphae originating deep within the subhymenial layer, 8-spored, operculum lateral; spores ellipsoid to fusoid, hyaline to slightly yellowish, 24–45 × 10–14 μ, usually containing conspicuous globules which vary in size and number, lying obliquely in the asci, sometimes slightly overlapped; paraphyses not flexuous, dichotomously branched several times, sometimes extending beyond the asci, when fresh filled with red coloring matter (said to be stained green in iodine solution when fresh).

On buried sticks and roots. Sweden, Norway, Finland, Switzerland, Hungary, Germany, Austria, England, Scotland, Russia, Japan, Canada, Moravia, United States.

MATERIAL EXAMINED: UNITED STATES. **Colorado**: May 4, 1897, D. M. Andrew, Ellis Coll., Bethel, *No. 238*, (*NY*). **Michigan**: Isle Royale, Nov. 15, 1941, Harold and Virginia Bailey, (*Mich.*). **Minnesota**: Hennepin Co., May 1903, Hibbard, Daisy Hone Collection, (*Minn.*).

CANADA. **Manitoba**: April–May 1925, A. H. R. Buller, (*Win.*).

ENGLAND: Dunkeld, Perths. Potter, [Spec. George Massee Herb. (*NY*)].

HUNGARY: Budapest, Flora Hungarica exsiccata *No. 610*, (*Mich.*); Budapest. F. Petrak, Mycotheca generalis *No. 36*, (*NY*).

Accounts in the literature show that this species is collected in cold seasons. Schroeter (**1908**) and Buller (**1934**) state that it has been collected on frozen ground, or after the first melting snow. Usually it is found from March to early May. The Isle Royale collection, of November 15, represents a late date for fungi at that latitude.

The genus *Microstoma* was established by Bernstein for a fungus found near Breslau, Germany. He presented his paper on *Microstoma hiemale* in 1851 and publication followed (**1852**). Immediately following his article, in the same publication Milde (**1852a**) published a brief account in which he commented approvingly on Bernstein's work. Again in the same year (whether before or after Bernstein's publication no one seems to be able to decide), Milde (**1852b**) wrote again concerning Bernstein's collection and another one made by Nees. In this account Milde made the error of assigning Nees and Bernstein as co-authors for the species, and this slip has led to confusion regarding author citation. There is no question, however, but that Bernstein should be credited with the publication of the genus and of the species *M. hiemale*. Milde did a special service in his second report for he extended the description of the structure of the fungus to include a good discussion of the gelatinous layer. He said that it was present in the stipes and could be traced definitely up into the cups. This gelatinous character was also noted by Lloyd (**1920**) in connection with a collection of this fungus from the Clinton Herbarium. Lloyd wrote concerning it, "if it is gelatinous as I thought it was, I do not see how it can be classed as *Sarcoscypha*." Seaver identified this collection as *Sarcoscypha cruciata*. A slide made from this material is in the New York Botanical Garden Herbarium, but, unfortunately, it is too old and dry for one to be able to distinguish the character of the hypothecium. An attempt was made to see

the remainder of this collection which, presumably, is in the Buffalo Academy of Science Herbarium. Due to the fact that the fungi in the Clinton Herbarium have not been catalogued, the specimen (if still in existence) is not available for study. Another North American collection which I examined was *Sarcoscypha alpina* E. & E. This material was collected in Colorado by Bethel. A part of a single small apothecium is all that is left of this collection in the herbarium of the New York Botanical Garden. It is not possible to say whether or not a gelatinous layer is present. The paraphyses appear to be dichotomously branched instead of filiform as reported by Ellis. The spores are right for *M. protracta.* A collection from England in the George Massee Herbarium is in too poor condition to allow examination. The collection in the Daisy Hone Herbarium is an alcoholic preparation, is badly fragmented, and it was impossible to make a satisfactory study of it. The Canadian material from Manitoba was from the location from which Buller had taken specimens for his exhaustive study on the mechanism of spore dispersal for this species (**1926, 1934**). A thin gelatinous layer was found. In the Isle Royale material and in the two collections from Hungary, the gelatinous layer was well developed. It was thick and easily separable from the two adjoining layers of hymenial tissue. The gelatinous tissue was found both in the stipes and cups just as Milde (**1852b**) had reported it. Gelatinous tissue is not reported for species of *Plectania,* and its presence is not mentioned in descriptions of *Sarcoscypha* spp. which have not yet been transferred to *Plectania.*

Another morphological character which is unique for this species is a pseudorhiza or root-like structure from which the aerial stipes arise. It is hard, somewhat finger-shaped, and dark in color. It apparently is perennial and probably facilitates fruiting in the winter season. Bernstein called it a rhizome but the term pseudorhiza proposed by Buller (l. c.) is the better term for it. Buller stated that it "corresponds exactly to the 'rooting base' of such Hymenomycetes as *Collybia radicata.*" He also stated that the second year's growth may develop a cluster of fruitbodies up to eleven in number, that this cluster may die away and a new set of fruitbodies appear which will persist until the third spring.

Heim (**1925**) has reported upon it as it is found in European collections.

The fungus has been found in both hardwood and coniferous woods. There is probably an attachment to tree roots. Gelin (**1938**) states that the "opinion is generally held that the fungus is a saprophyte, growing on pieces of decomposing branches and roots of different shrubs and trees, Larix, Acer, Corylus, *Populus tremula* etc."

Still a third character which calls for comment is the paraphyses. They are branched dichotomously and form compact, erect bundles. At certain stages in the development of the asci they project beyond the tips of the asci, according to Buller, and this can sometimes be observed in slides of herbarium material. Bernstein described the paraphyses as branched but, unfortunately, he did not illustrate them. They are not filiform.

The genus *Microstoma,* as described by Bernstein and extended by Milde, was established primarily upon the presence of gelatinous tissue and pseudorhiza. These, together with the type of paraphyses, warrant the transfer of *Plectania protracta* to *Microstoma.*

Peziza mirabilis, which was described by Borszczow (**1857**) from a collection made in Russia, is known only from his account. It is generally considered to be synonymous with *M. protracta,* but it needs further study. He did not report the presence of any gelatinous layer. His illustration of the paraphyses shows them to be rather stout, filiform and septate.

In connection with the use of the name *Microstoma,* a situation exists that needs clarification. Seaver (**1928**) in his synonymy of *Plectania* cited "Not *Microstoma* Bruch. 1846" which implies that the name had been used as a genus preceding Bernstein's publication. Upon investigation it was found that the name *Microstoma* as used by Bruch designated a section, not a genus, and therefore does not pre-empt the use of the name by Bernstein. Pfeiffer (**1874**) gives "*Microstoma* Bruch, Schimp. & Gümb. 1846. Bryol. eur. Fasc. 33–36. sect. Hymenostomi (*H. microstomum et squarrosum*)." In other words it is not a synonym. The question of the validity of *Microstoma* as a generic name, as used by Bernstein, was submitted to Dr. G. R. Bisby, who gave the above solution, which agrees with that of the writer.

There is also a question of nomenclature regarding the use of the specific name *protracta* that calls for discussion. Fries (**1851**) published descriptions of two fungi that had been collected in Sweden. He named them *Peziza cruciata* and *P. protracta* in the order named. Gelin (**1938**) and Buchwald (**1941**) state that it is a generally accepted fact that these two fungi are identical. Buchwald states that the type of *P. protracta* no longer exists, and that *P. cruciata* is in the Herbarium at Uppsala. Gelin in establishing the combination *Plectania protracta* used the prerogative provided for in the International Rules of Botanical Nomenclature (Briquet **1935**) and chose the specific name *protracta* instead of *cruciata*. This was done, apparently, on the basis of *protracta* being the name in more common usage. Gelin's decision must be accepted and the valid combination, using the genus *Microstoma*, is the one proposed here.

ANTHOPEZIZA Wettstein

Verhand. Zöol.-bot. Gesells. **35**: 383. 1886.

Apothecia cespitose, subglobose becoming deep cup-shaped, externally hairy, hymenium scarlet; stipes long, slender, flexuous, hairy, sometimes branched; asci cylindrical, 8-spored; spores large, one-celled, smooth, hyaline to slightly yellowish; paraphyses repeatedly branched, anastomosing and forming a reticulum. No blue coloration with iodine.

Type: *Anthopeziza Winteri* Wetts.

The following is quoted from Wettstein (l. c.):

"Thalamia caespitosa, magna, longe stipitata, cum stipite flexuoso cornu speciem referentia, superne in cupulam dilatata, e mycelio denso nigrescente (non sclerotio) orta, carnosa, extus imprimis in parte inferiore lanato-pubescentia. Cupula campanulata, margine magis minusve regulariter fisso. Hymenium colore laeto. Asci longissimi, octospori. Paraphyses tenues, numerosae, apice clavatae, inter se irregulariter reticulatim connectae vel ramosae. Sporae maximae unicellulares enucleatae, 3–4 guttulatae.—Fungi terrestres, vere primo thalamia proferentes."

Anthopeziza floccosa (Schw.) comb. nov. (FIGS. 1–2, 10–12).

Peziza floccosa Schw. Trans. Am. Phil. Soc. II. **4**: 172. 1834.
Sarcoscypha floccosa Sacc. Syll. Fung. **8**: 156. 1889.
Geopyxis floccosa Morgan. Jour. Myc. **8**: 188. 1902.
Plectania floccosa Seaver. North Am. Cup-fungi, p. 192. 1928.

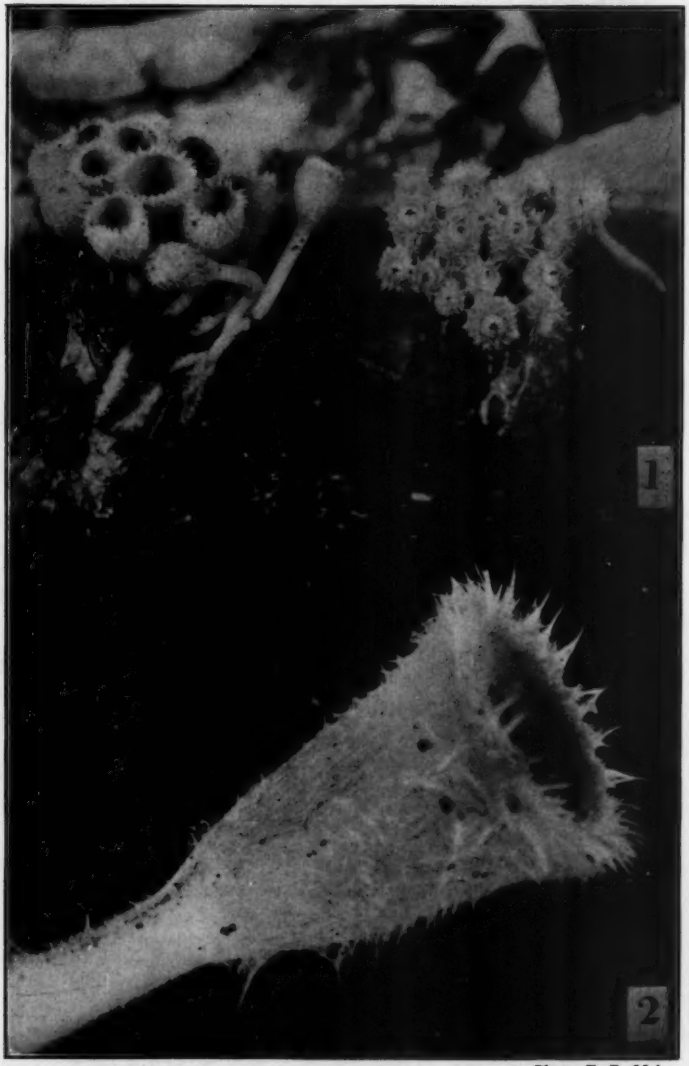


Photo. E. B. Mains.

FIGS. 1–2. *Anthopeziza floccosa.*

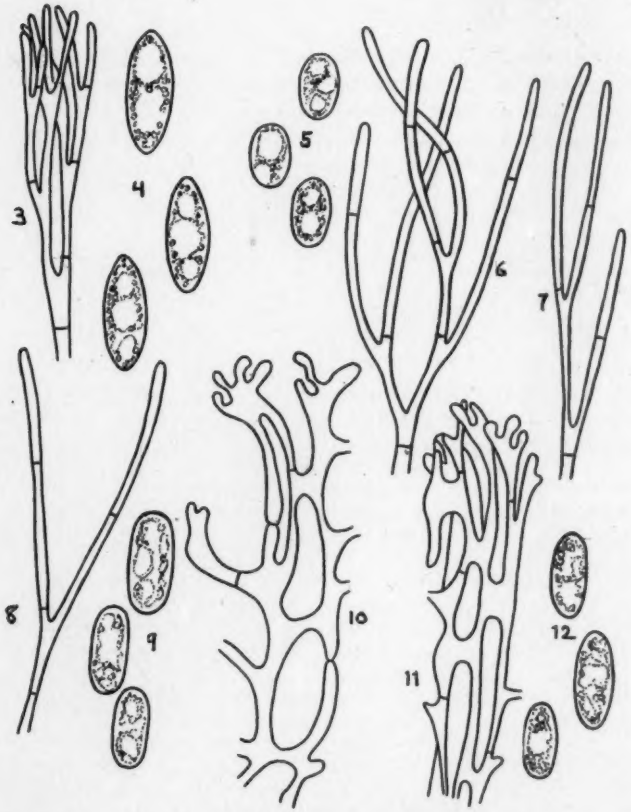
Apothecia stipitate, usually cespitose arising from buried sticks, frequently in clusters of from three to six fruitbodies, 0.5–0.75 cm. in diameter, 1–1.25 cm. in depth, subglobose, becoming inverted cone-shaped when mature, substance thin, fragile when dry, clothed with long, hyaline hairs which in the young stage form a stellate pattern over the upper portion; stipe slender, length variable up to 5 cm., covered with hyaline hairs, the lower portion dark colored, base frequently surrounded by a loose mass of hyphae; asci cylindrical, narrowed abruptly into a long stem-like base, 300–350 × 18–20 μ; spores hyaline, smooth, 20–35 × 14–16 μ; paraphyses branched and anastomosed forming a reticulum around the asci, small knoblike branches frequent, septate, slightly constricted at the septa, apices ending in branches that in some stages overreach the asci. No blue coloration with iodine.

Type locality: Nazareth, Pennsylvania. Distribution, United States.

MATERIAL EXAMINED: UNITED STATES. **Connecticut**: Norwich, July 4, 1890, W. A. Sturgis, (*NY*); Redding, July 19, 1902, Earle, *No. 495*, (*NY*). **Indiana**: Lafayette, July 13, 1930, G. B. Cummins, *No. 121*, (*NY*); Corydon, June 17, 1935, F. J. Hermann, (*Mich.*). **Kentucky**: Bowling Green, June 1896, I. F. Price (Herb. of Lucien M. Underwood), (*NY*). **Maryland**: Catoctin, June 8, 1940, J. I. Wood, (*BPI*). **Michigan**: Ann Arbor, July 4, 1905, C. H. Kauffman, (*Mich.*); Ann Arbor, July 2, 1916, E. B. Mains, (*Mich.*); Ann Arbor, H. M. Fitzpatrick and G. H. Smith, (*NY*); Ann Arbor, July 16, 1928, Gordon L. Walls, (*Mich.*); Ann Arbor, July 10, 1929, B. B. Kanouse, (*Mich.*); Pinckney, June 27, 1935, A. H. Smith, (*Mich.*); Chelsea, June 1937, A. H. Smith, (*Mich.*); Chelsea, June 27, 1937, A. H. Smith, *6411*, (*Mich.*); Waterloo Area, June 26, 1945, A. H. Smith, *20480*, (*Mich.*); Moscow, August 15, 1947, Bryant Walker Coll., (*Mich.*). **Minnesota**: Waseca Co., July 1891, Sheldon, *No. 667*, Daisy Hone Herb., (*Minn.*); Winona, June 27, 1895, J. M. Holzinger (Ellis Coll.), (*NY*); Groveland, July 1904, Em. Freeman (Daisy Hone Coll., *No. 3103*), (*Minn.*). **Missouri**: Perryville, 1884, C. H. Demetris (Rabenhorst-Winter, Fungi Europaei, *3171*), (*NY*); Columbia, June 25, 1940, J. B. Routien, *No. 1156*, (*NY*). **New Jersey**: Newfield, Aug. 2, 1881, Ellis Collection, (*NY*). **New York**: Jamesville, June 1889, L. M. Underwood, (*NY*); Greenbush, Ellis Coll., (*NY*); Jamesville, June 1889, L. M. Underwood and O. F. Cooke, *No. 79*, (*NY*); Carrollton, Sept. 1902, C. H. Peck, (*NY*); Greenwood, July 6, 1930, W. S. Thomas, (*NY*); Long Island, Zabristne (Ellis Coll.), (*NY*); Herbarium of W. R. Gerard (*NY*). **Ohio**: Oxford, July 1, 1909, Bruce Fink, (*Mich.*); A Lloyd Coll. in Ellis Coll. (Ellis Coll.), (*NY*); Seven Caves, June 5, 1935, W. B. Cooke, *No. 5036*, (*NY*).

Pennsylvania: Nazareth, Ex-Schweinitz Herbarium (ex-Michener Herb.), (*BPI*), slides of *type* material; Nazareth, Schweinitz Herb., (*PH*); West Chester, Aug. 1879, Rev. M. T. Jefferis N.A.F., *No. 435*, (*NY*); West Chester, August 1879, Haines and Everhart (Ellis, *No. 822*), (*NY*);

Nazareth, June 28, 1883, ex herb. E. A. Rau, (*BPI*); Bethlehem, August 1884, E. A. Rau Rabenhorst-Winter, Fungi Europaei, *No. 3171* Supplement, (*NY*); West Chester, June 1889, B. M. Ellis N.A.F., V (*NY*); Ohio Pyle, July 3–8, 1905, W. A. Murrill, (*NY*). **Tennessee**: Knoxville, June 1928, U. Tenn. *No. 2101*, (*Mich.*); La Follette, July 11, 1934, L. R. Hesler,



FIGS. 3–11. Spores and paraphyses of *Microstoma, Plectania* and *Anthopeziza*.

(*Mich.*); New Hopewell, May 26, 1938, A. J. Sharp, (*BPI*). **West Virginia**: Fayette Co., July 12, 1893, L. W. Nuttall, *No. 1112* (*Mich.*). **Wisconsin**: Madison, 1903, R. A. & A. M. Harper (Rehm: Ascomyceten, *No. 1776*), (*NY*); Kewaunee Co., B. O. Dodge, *No. 116*, (*NY*); Madison, 1903, R. A. & A. M. Harper (Rehm: Ascomyceten, *No. 1776*), (*Mich.*).

Wettstein's account of the reticulate condition of the paraphyses in the genus *Anthopeziza* has been overlooked or disregarded. Some descriptions have even been altered to record the paraphyses as filiform. This has led to erroneous interpretations regarding *A. Winteri* which are to be found in several lists of synonymy. The finding of a reticulate condition in the paraphyses of *Peziza floccosa* emphasizes the importance of Wettstein's observations and lends weight to the validity of his genus. The two species *A. Winteri* and *A. floccosa* are not identical. In *A. Winteri* the paraphyses are described as having clavate tips, and they are illustrated as having a simple, regular type of reticulum. In *A. floccosa* the anastomosis is complicated and irregular, and the tips are finely branched. Wettstein's drawing shows the apothecia to be less hairy than are the cups of the North American species, *A. floccosa*. The beautifully arranged hairs over the exterior of the cups of *A. floccosa* (FIGS 1–2) are a conspicuous feature and help in spotting that fungus in the field. It is possible, of course, that *A. Winteri* is but a depauperate form of *A. floccosa*. It is desirable that further study be made on the European species. A careful search might show that the European species grows in North America. *A. floccosa,* as it was represented in the collections examined for this study, was found to be a remarkably clear-cut and distinct species with almost no variation in its morphological characters.

ANTHOPEZIZA WINTERI Wettstein, Verhand. Zöol.-bot. Gesells. **35**: 383. pl. 16. *figs.* 1–7.

Copied from Wettstein:

Thalamia 2–10, consociationes e mycelio communi subterraneo, nigrescente, denso ortae. Thalamium initio curvato-clavatum, apice clausum; deinde longum, clavato-cornuforme, stipite duro; curvato, superne, in cupulam apertam dilatato, extus lanato-pubescenti, 3–5 cm. longum, non plicatum. Cupula initio globoso-campanulata, ore orbiculari (formam floris Convallariae maialis fere referens) extus glabra vel parce puberula, pallide aurantiaca, margine regulariter in dentes 8–12 subreflexos, extus parce pilosos fisso; deinde multo accrescens, circa 2 cm. longa, 1½ cm. diametro, campanulata, extus glabra, lobis marginis trigonis reflexis magnis. Hymenium intense cinnabarinum, partem interiorem cupulae margine pallido excepto obtegens. Cupula demum non explanata, saepe irregulariter lacerata (imprimis aere humido). Asci longissimi, cylindracei, hyalini, 0.4–0.7 mm. longi, 12–16 Mikromm. diametro,

apice rotundati. Paraphyses tenues, circa 0.4–0.7 mm. longae, apice clavato-incrassatae et extus verruculis minimis obsitae, rarius indivisae, plerumque ramosae vel inter se ramulis tenuissimis connectae itaque fasces densas inter ascos formantes, in parte superiore oleo rubro intense colorato tinctae. Sporae octo, in parte superiore asci, oblique monostichae, ellipticae vel (rarius) elliptico-oblongae, hyalinae, glabrae, membrana crassa, vacuolis tribus vel rarius quatuor ficte du-vel triseptatae, unicellulares, 33–35 Mikromm. longae, 11–13 Mikromm. latae.

Austria inferior. In locis umbrosis ad silvarum margines valleculae, "Oeder Saugraben" prope Rodaun; mense Martio ad nives liquescentes.

Wettstein transferred *Sclerotinia baccata* Fuck. to *Anthopeziza*. Rehm (**1895**) placed it in synonymy with *Sarcoscypha protracta*. Fuckel's illustration is of little help. Just where *S. baccata* belongs I am not prepared to state.

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EXPLANATION OF FIGURES

FIGS. 1–2. Photographs of *Anthopeziza floccosa*. Photo. E. B. Mains.

FIGS. 3–4. *Microstoma protracta;* 3, a paraphysis showing dichotomous branching; 4, spores.

FIGS. 5–7. *Plectania occidentalis;* 5, spores; 6–7, branching paraphyses.

FIGS. 8–9. *Plectania coccinea;* 8, branching paraphysis; 9, spores.

FIGS. 10–12. *Anthopeziza floccosa;* 10–11, anastomosing paraphyses showing the reticulum surrounding the asci; 12, spores.

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NOTES AND BRIEF ARTICLES

HUMARIA AND LACHNEA. When several years ago an attempt was made by the writer to monograph the operculate cup-fungi of North America much difficulty was encountered in deciding upon the proper generic names to be used in many cases. Probably no two names were more difficult to unscramble than *Humaria* and *Lachnea* both of which were in general but illegitimate use at the time.

The name *Humaria* was first used by Fries (Syst. Myc. **2**: 42. 1922) as a section or subgenus of *Peziza* and applied to a group of the smaller, nonvillose discomycetes. In 1889 Saccardo (Syll. Fung. **8**: 118) raised Fries' subgenus to generic rank including a number of the species originally included by Fries in the subgenus. Unfortunately, however, in the meantime Fuckel (Symb. Myc. 320. 1869) obviously appropriated Fries' name but attributed it to himself, as he had done in other cases (see *Peziza* Fuckel, Symb. Myc. 306), and used it in an entirely different sense, applying it to the villose or hairy forms placed by Fries in the series or subgenus *Lachnea*. Therefore Saccardo's use of the name was invalid. The writer realizing this and in order to cause as little confusion as possible proposed *Humarina* to replace the untenable name *Humaria* of Saccardo.

The name *Lachnea* was also found to be untenable since it had previously been used for a genus of flowering plants. This was replaced by the name *Patella* of Weber in 1780, *Humaria* of Fuckel (not *Humaria* of Saccardo) being cited as a synonym of *Patella*.

Now after two decades, Dr. Bessie Kanouse of Michigan has discovered that *Patella* is untenable under the International Rules, although perfectly tenable under the American code which was in use at the time this monograph was written, and concludes that *Patella* must be replaced by *Humaria* of Fuckel. If her suggestion were to be followed, we would be placed in the embarrassing and rather ridiculous position of having the same name applied to two different genera of the discomycetes, *Humaria* (Fries) Sacc. and

Humaria Fuckel. Such a suggestion could serve no useful purpose but only add to the confusion which has already existed in the disposition of these two names. To back up her decision, eight new combinations have been proposed by her (*Mycologia* **39**: 655, 656. 1947). Incidentally two of these combinations were not new at all but had been previously used and were cited as synonyms in North American Cup-fungi.

Regardless of these facts, Dr. Kanouse states "According to the International Rules of Nomenclature the name *Humaria* [of Fuckel] must be used for the species commonly relegated to the genus *Lachnea*." But must it? The same International Rules state (Sec. 2, Art. 62) "A name of a taxonomic group must be rejected if owing to its use with different meanings it becomes a permanent source of confusion or error." In our opinion this latter rule fits the case in question perfectly.

Whatever name is used to replace the untenable name *Lachnea* the use of the name *Humaria* of Fuckel cannot be justified under the International Rules. While there is nothing to prevent anyone from using any name he or she may choose it is suggested, in the light of the above facts, that the name *Humaria* be allowed to continue to quietly slumber in synonymy where it was placed, and we believe for good and valid reasons, twenty years ago.—FRED J. SEAVER, THE NEW YORK BOTANICAL GARDEN.

SOME FUNGI COMMON TO THE HIGHLANDS OF MEXICO AND GUATEMALA AND EASTERN UNITED STATES.[1] While the writer[2] was doing botanical field work (June 1944 to May 1946) in Mexico and Guatemala, indiscriminate collections were made of the easily preserved fungi. About half the collections have been identified[3] and of these about one third are identical with species of eastern United States.

In addition to those dried and preserved in the Herbarium of The University of Tennessee, the following species were noted in the

[1] Contributions from The Botanical Laboratory, The University of Tennessee, N. Ser.

[2] As a Fellow of the John Simon Guggenheim Memorial Foundation.

[3] For which thanks are due Dr. L. R. Hesler, Dr. W. A. Murrill, Dr. J. A. Stevenson and Dr. J. A. Miller.

LIST OF SOME FUNGI OCCURRING BOTH IN THE HIGHLANDS OF MEXICO AND GUATEMALA AND IN EASTERN UNITED STATES:

PYRENOMYCETES

No. of Collections*	Fungus	Distribution as Previously Listed†
1	*Hypoxylon malleolus* Berk. & Rav.	Trop. Amer. up to Miss.
1	*Xylaria cubensis* Mont.	S.E. U.S. & Cuba

BASIDIOMYCETES

Thelephoraceae

2	*Aleurodiscus candidus* (Schw.) Burt	E. U.S. to Cal.; Mex. & Jamaica
1	*Stereum ochraceo-flavum* (Schw.)	E. U.S. to Cal.; Mex.
1	*Stereum rameale* Schw.	N.A. to Mex.; Jamaica & Puerto Rico
2	*Stereum sepium* Burt	E. U.S.; Mex.; Mass. & Wisc. to D.C. & Missouri; Colombia
1	*Tremellodendron merismatoides* (Schw.) Burt	

Polyporaceae

1	*Irpex farinaceus* Fr.	Trop. Am. & N. to Ohio and Iowa
7	*Polyporus abietinus* Fr.	N.A., Eur. & Asia
10	*Polyporus adustus* Fr.	Cosmop.
7	*Polyporus arcularius* Fr.	Conn., Fla. to Colo. & Mex.
1	*Polyporus australis* Cooke	S. U.S., trop. Amer. & trop. Asia
2	*Polyporus cinnamomeus* Fr.	Cosmop.
1	*Polyporus cristatus* Fr.	N. Central states
3	*Polyporus Curtisii* Berk.	N.Y. to Fla. & W. to Texas
1	*Polyporus cuticularis* Fr.	N. Central states
4	*Polyporus dichrous* Fr.	W. to Mo. & Kansas
1	*Polyporus distortus* Fr.	Can. & U.S. W. to Wisc. & Texas
7	*Polyporus gilvus* Fr.	Cosmop.
15	*Polyporus hirsutus* Fr.	N.A., Eur. & Asia
2	*Polyporus licnoides* Mont.	Trop. Amer., Gulf states to Missouri
1	*Polyporus lucidus* (Leyss.) Fries	Gulf states to Conn. & Minn.
2	*Polyporus perennis* Fr.	Temp. reg. of world; in U.S. south to Va.
4	*Polyporus pinsitis* Fr.	S. Fla. & Mex. to Brazil
1	*Polyporus pocula* (Schw.) Berk. & Curt.	Ohio, Tenn., and West to Missouri
1	*Polyporus rhipidium* Berk. (*Favolus rhipidium* Berk.)	Ohio to Wisconsin
1	*Polyporus semipileatus* Pk.	E. U.S.; Maine-Fla.

* To give a crude idea of relative abundance.

† For most of the geographical information concerning the distribution of the fungi listed below, reference was made to the treatment of the Polyporaceae by Overholts, Murrill's work in the North American Flora and Burt's Treatment of the Thelephoraceae, all admittedly out of date.

BASIDIOMYCETES—*Continued*
Polyporaceae—Continued

No. of Collections*	Fungus	Distribution as Previously Listed†
4	*Polyporus sanguineus* Fr.	Tropical reg. of world
1	*Polyporus Schweinitzii* Fr.	N.A., Eur. & Asia
1	*Polyporus sulfureus* Fr.	Cosmop.
7	*Polyporus tulipiferus* (Schw.) Overh.	N.A., Eur. & Asia
18	*Polyporus versicolor* Fr.	Cosmop.
8	*Fomes annosus* (Fr.) Cooke	N.A. & Eur.
4	*Fomes Feei* Fries	Fla. & trop. Amer.
9	*Fomes pinicola* Cke.	Temp. regions
1	*Fomes roseus* (Alb. & Schwein)	N. Amer. & Eur.
1	*Fomes senex* Mont.	Trop. Amer., Africa, Asia and S.E. U.S.
1	*Trametes malicola* B. & C.	Can. & N. Cen. states
5	*Trametes sepium* Berk.	Temp. N.A.
1	*Daedalea quercina* L. ex Fr.	Temp. N. Amer. & Eur.
8	*Lenzites betulina* Fr.	Temp. N. Amer. & Eur.
6	*Lenzites saepiaria* Fr. (*L. Berkleyi* Sacc.)	N. Temp. zone
	Agaricaceae	
3	*Schizophyllum commune* Fr.	Cosmop.
1	*Pleurotus ostreatus* Fr.	Temp. N.A.
1	*Lactarius indigo* (Schw.) Fr.	E. U.S.; Vt.-Fla.
	Nidulariaceae	
1	*Crucibulum vulgare* Tul.	U.S. & New Zealand
	Calostomaceae	
4	*Calostoma cinnabarina* Desv.	Miss. to Penna.

field or markets: *Amanita caesaraea, A. chlorinosma, A. muscaria, A. rubescens, A. verna; Amanitopsis vaginata* var. *fulva* and the var. *livida; Cantherellus cibarius, C. floccosus; Clitocybe laccata; Collybia platyphylla; Lactarius Peckii; Lepiota procera;* and *Russula foetens.*

In the list presented below, some species are recorded as cosmopolitan, *e.g., Polyporus adustus, P. cinnamomeus, P. gilvus, P. sulphureus, P. versicolor* and *Schizophyllum commune.* With the possible exception of the last-named species it is my belief that they are not truly cosmopolitan. In Guatemala and Mexico they seem to be limited to the temperate regions and were not encountered on my infrequent excursions into "hot country."

Some species, *e.g., Irpex farinaceus, Polyporus australis, P. pinsitis* and *P. sanguineus,* seem to have their greatest distribution in the tropics, and probably eastern United States represents the fringe of their ranges.

Other species listed above, *e.g., Polyporus cristatus, P. cuticularis, P. semipileatus, Fomes pinicola, Trametes malicola, Lenzites betulina, Pleurotus ostreàtus* and *Lactarius indigo,* have been reported seldom or not at all from south of the United States. That these and many other fungi common to eastern United States should also be growing in the temperate regions of Mexico and Guatemala is not surprising. In these Latin American highlands are many vascular species identical with, or closely related to, plants in eastern United States. Pine and oak forests, both pure and mixed with each other, abound. In addition, in these and in very heterogeneous, mixed temperate forests occur such species as: boxelder, beech, sourgum, sweetgum, redbud, basswood, sugar maple, blue beech (*Carpinus*), hophornbeam (*Ostrya*), elm, ash, holly, alder, sycamore, storax, buckthorn, dogwood, hawthorn, elderberry and wild black cherry. Other trees closely related to northern species may be found. Thus are provided living and dead substrata equal to those of eastern United States.

Moreover, between the elevations of 4,000 and 7,000 feet where most of the observations were made, the temperature extremes are those of the season in which fungi put in their most prolific appearance in eastern United States. Freezing weather is rare and on the warmest days a thermometer will seldom register over 90° F. From May to November there is usually an abundance of rain and a relatively high humidity. Thus, the substrata and the climate during half the year are such as would favor the growth of many of the fungi reported from eastern United States.

AARON J. SHARP, UNIVERSITY OF TENNESSEE.

GRAMINICOLOUS SMUTS OF NORTH AMERICA. Century I.

In the preparation of the new exsiccati series "Graminicolous Smuts of North America," I have the objective of distributing among certain institutions throughout the world authentic and representative specimens of smut fungi on grasses and cereals collected in North America. The species concept used in the naming

of these specimens is based on that used in recent published articles on graminicolous smuts by myself and co-authors. Requests have been received for illustrative specimens of several controversial species to the extent that this new exsiccati series would seem to find welcome in various herbaria throughout the world.

Most of the specimens in the first Century have been collected by myself or various loyal co-workers, and represent largely the Pacific Northwest. It is expected that future centuries will include to a greater extent other regions of the United States, as well as Canada and Mexico.

The preparation of this exsiccati series is a cooperative project, involving the Washington Agricultural Experiment Station, Pullman, Washington; and the Divisions of Mycology and Disease Survey and Forage Crops and Diseases, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture. With a view toward wide geographic distribution, complete sets are being deposited in the following herbaria: Mycological Collections, Bureau of Plant Industry, Beltsville, Md.; Department of Plant Pathology, Washington State College, Pullman, Washington; Farlow Cryptogamic Herbarium, Cambridge, Mass.; New York Botanical Garden, New York; University of Michigan, Ann Arbor, Mich.; University of California, Berkeley, Calif.; Division of Botany and Plant Pathology, Central Exper. Farm, Ottawa, Canada; Spegazzini Botanical Institute, La Plata, Argentina; Commonwealth Mycological Institute, Kew, England; Institute of Systematic Botany, University of Uppsala, Uppsala, Sweden; Museum of Natural History, Paris, France; Institut de Botanique et Herbier Boissier, Geneva, Switzerland; Herb. Crypt. Ind. Orientalis, New Delhi, India; Department of Agriculture, Sydney, New South Wales.

I should be very grateful to receive collections of graminicolous smuts, especially on grasses, from any part of North America in sufficient quantity for inclusion in this exsiccati series. Full credit will be given to the collectors in every instance.—GEORGE W. FISCHER.

LABORATORY DIAGNOSIS OF MYCOTIC DISEASES

1. A refresher course for laboratory personnel in the Laboratory Diagnosis of Mycotic Diseases will be offered at the Laboratory Division of the Communicable Disease Center. The first course will be given from August 30 to September 24, 1948.

2. This training is open to all grades of employed laboratory personnel. Although first consideration will be given to the laboratories of state and local public health departments, applicants from hospitals and private laboratories will be considered when vacancies occur.

3. There is no tuition or laboratory fee but travel and living expenses must be paid for by the individual or his employer.

4. Applications for the course should be made as far in advance as possible. Notification of acceptance from this office will be made in sufficient time to allow the students to make arrangements for living accommodations. It is suggested that trainees obtain reservations for living accommodations at the earliest possible date. A list of hotels and rooming houses will be sent to applicants at the time of acceptance.

TENTATIVE OUTLINE FOR THE FOUR-WEEKS COURSE IN THE LABORATORY DIAGNOSIS OF MYCOTIC DISEASES

Phases of the program and relative amount of time allotted to each is as follows:

1. *Identification of common saprophytes* — 3 days
   Aspergillus, Penicillium, Cephalosporium, Fusarium, etc.
2. *Identification and culturing of the dermatophytes* — 5 days
   Trichophyton, Microsporum, Epidermophyton, etc.
3. *Identification and culturing of the sub-cutaneous fungi* — 6 days
   Hormodendron, Phialophora, Sporotrichum, Allescheria, Nocardia, Actinomyces, etc.
4. *Identification and culturing of the systemic fungi* — 6 days
   Coccidioides, Histoplasma, Blastomyces, Cryptococcus, etc.

Stress will be placed on practical laboratory procedures useful for establishing a diagnosis of mycotic infection, including the following:

1. Isolation techniques.
2. Preparation and use of special culture media.
3. Fermentation reaction tests.
4. Vaccine preparation.
5. Agglutination and complement fixation tests.
6. Inoculation of animals.
7. Preparation of permanent mounts.
8. Slide cultures.

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